Clinical Research

PROCEEDINGS

ficial Publication of The American Federation for Clinical Research

VOL. V, NO. 1 JANUARY, 1957

IN THIS ISSUE

Published by Grune & Stratton, Inc.

Vol. V, No. 1 Clinical Research PROCEEDINGS January 1957

Back to Harvey

By E. E. Eddleman, Jr.*

Because of the enormous amount of time and money being spent today on cardiological research, it seems appropriate that the general direction and scope of current and past investigation of this subject be re-evaluated. The question can properly be asked, "Are time, money, and interest being directed along lines where information is needed most?" The following discussion does not attempt to answer fully this question, but rather points out some aspects of a neglected and important phase of cardiovascular research.

It is understandable that investigation of the cardiovascular system has progressed primarily with the development of technics for studying the heart and circulation. To a certain extent, experimentation has not always been planned to obtain more needed knowledge; instead, it has often been determined by the availability of technics and instruments. In some instances, progress has been orderly and rationally achieved; in others, however, it has not. Many technics currently in use have not necessarily been selected or developed to fill a need in solving a specific problem but have been adapted for use before ideas were conceived. Thus, possibly the most important aspect of cardiological research—the problem of measuring cardiac function and reserve (cardiac mechanics)—remains essentially unexplored.

Actually, one may divide the history of cardiovascular research into several major eras. The first, or *descriptive era*, dates back to the early anatomists who had available for study only gross specimens obtained from autopsy material. From this period came the near perfect descriptions of the heart.

The second may be termed the *mechanical era*. This era just preceded the development of modern technics, and therefore interest was directed toward defining gross cardiac dynamics, mechanics of circulation associated with the flow of blood, and movements of the heart. By far the most outstanding work of this period was that done by Sir William Harvey, who first described the circulation and movements of the heart.[1] It is remarkable how accurately cardiac dynamics were depicted. Actually, up to the present day very little has been added to his original description, and it is obvious that great gaps exist in current knowledge of cardiac dynamics.

The third, or *physiologic era*, was ushered in with the development of technics for measuring pressures, changes in heart volume, and blood flow. Such early investigators as Starling contributed a great deal of information concerning the influence of inflow and outflow loads and changes in diastolic length of the myocardial fiber on the over-all function of the heart. This work was climaxed by the fundamental and now classic studies of Wiggers, Hamilton, and others in defining the cardiac cycle, pulse contour, pressure pulses, and cardiac output. This era was largely an era of dog experimentation, while comparable studies in human subjects were delayed until the development of right and now left heart catheterization.

The present era is marked by the simultaneous development of three approaches—electrocardiographic, catheterization and metabolic—which make up the bulk of the cardiovascular research now being pursued. Contribution to knowledge and clinical cardiology from the electrocardiographic studies is obvious; however, the one important and frequently overlooked defect in this approach to the study of circulation is that it does not give information about mechanical heart function or reserve. Many examples are routinely noted in which abnormal electrocardiograms are associated with a normally functioning heart and, conversely, many instances of marked cardiac impairment occur in the presence of normal electrocardiograms. Catheter studies in the human heart have largely confirmed, modified, or amplified already existing data obtained in the experimental animal during the earlier eras. (This applies to basic knowledge, since the clinical value of the technic, especially in cases of congenital heart disease, is undisputed.) In addition, it has become increasingly apparent that the absolute level of cardiac output is of minor importance because, generally speaking, it gives little information as to "how" the heart achieves a given output. Many clinical examples have been reported and continue to appear in which the cardiac output is normal or even elevated in the presence of congestive heart

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failure. Modern biochemical methods have led to rapid exploitation of the metabolic aspects associated with heart disease, the importance of which is apparent. Studies on myocardial metabolism fail to yield any significant difference in metabolic pathways for the failing heart, as compared to the normal heart,[2] except in such instances as beri-beri heart disease. However, recent studies on the nature of the contractile proteins of the heart muscle have indicated a difference in structure associated with the failing heart.[3] In addition, it is quite likely that the estimates of cardiac efficiency, which are based primarily on cardiac output and food consumption or on oxygen utilization, are not accurate, since it is now apparent that the metabolic pathways of the heart are both anaerobic and aerobic.[4] Also, these calculations do not take into consideration the forces that are expended in producing the movements of the heart, which in abnormal conditions may be appreciable. (These abnormal movements will be discussed in more detail subsequently.) Biochemical methods do not furnish either an accurate index of efficiency or a clue to the derangement of cardiac function; therefore, attention must be directed toward a mechanical rather than a metabolic approach in an attempt to solve existing problems in cardiac reserve.

The obvious flaw in cardiovascular investigation at the present time is the deficiency in knowledge of the mechanics of circulation. The crucial point is to know how well the heart is able to function and how well it can perform the tasks imposed upon it in daily living. Similarly, it is not nearly so important from a fundamental point of view to know how much blood the heart is ejecting in a minute as it is to know the mechanics, efficiency and reserve of heart function by which this is accomplished. At the present time there are only a few methods by which the mechanical function of the heart can be approached, other than from the historical evidence of how much activity the patient is able to tolerate. The concept of Sir James Mackenzie that the test of the heart is its response to effort[5] is still the most reliable guide in evaluating cardiac reserve.

It seems likely that an understanding of the factors associated with the functional reserve of the heart lies in the elucidation of the mechanics associated with ventricular contraction and relaxation. The process of ventricular contraction and relaxation is extremely complicated; it is not a matter of just a simple squeeze of the ventricles to achieve ejection. So little is known that it is difficult to develop any organized concept; however, there is some information which bears on the subject. All portions of the heart contract, not simultaneously but sequentially, beginning in certain portions, spreading to the remainder of the heart in an orderly fashion, and reaching a peak during early ejection. This fact can be noted through careful observation of the heart in situ and also in the photographic evidence of an orderly spread of the contractile process,[6] probably beginning in the inflow tract of the right ventricle and spreading to the outflow tract, the last portion contracting being the upper posterior and lateral aspect of the left ventricle. Since recent evidence has shown that most of the heart, or at least the inner two-thirds of the entire ventricular musculature, is excited almost instantaneously,[7] it is obvious that there is a varying electromechanical lag in the various parts of the heart. But again, there are almost no studies of factors concerning this electromechanical lag and why one portion of the heart, although electrically excited synchronously with other parts, is relatively delayed in its onset of contraction.

It is quite possible that relaxation similarly proceeds in an orderly fashion; however, whether the parts of the heart which are activated first relax first, or whether the relaxation process spreads in the reverse fashion, has not been demonstrated. The process of contraction is apparently highly coordinated and efficient since the shape-changes during isometric contraction increase the transverse diameter of the heart and place the bolus of blood in a much more favorable position for ejection. Thus, by centrally shifting the blood directly within the circular, thicker, and more powerful myocardial fibers, a more efficient expulsion can be achieved. This shape-change (possibly the result of contraction of the apical region of the heart and papillary muscles) is important; the musculature is much thinner in the apical region than it is at the base, and unless it is contracted initially the more powerful circular fibers around the base of the heart tend to neutralize its contraction. It is quite likely that disproportional hypertrophy of the various sections of the heart, as well as loss of muscle due to myocardial infarction, can seriously alter the systematic and orderly contractile process, thus interfering appreciably with heart function.

Evidence is being accumulated to show that the exaggerated apex thrust in left ventricular hypertrophy is largely the result of an aneurysmal bulge, probably due to the excessive strength of the basilar fibers of the heart in comparison to the thinner walled apex. Thus, during contraction, the thin wall portion balloons out in a manner similar to that frequently noted in the area of a myocardial infarction. This could conceivably divert and trap blood which would normally be ejected and consequently interfere appreciably with the efficiency of the contraction. Again, unilateral hypertrophy of the right ventricle apparently disturbs the normal contractile process considerably. The commonly palpable precordial "heave" clinically detected in most instances of right ventricular hypertrophy would, of necessity, have to be "the result of" a very strong force in order to lift the precordium as it often does. It is thought that this "heave" is caused by an anterior movement of the heart and pre-

cordium, resulting from the increased force of contraction of the hypertrophied right ventricular fibers.

It is extremely interesting to note that the response of the myocardium to abnormal loads can conceivably depend upon disproportionate hypertrophy of the various portions of the heart. It is well known that conditions associated with symmetrical or equal loading of the myocardium, such as A-V fistulae and thyrotoxicosis, are extremely well tolerated by a normal heart for a long time; in fact, many observers believe that patients with thyrotoxicosis may never develop heart failure without underlying myocardial disease. This is in contrast to influences which load the heart asymmetrically, such as pulmonary hypertension, systemic hypertension, aortic insufficiency and other valvular diseases, which in general may result in early deterioration of myocardial function. It is tempting to speculate that as long as the ventricles are loaded symmetrically, the contractile process is not distorted but remains exceedingly efficient and can tolerate enormous amounts of stress or work over a long period of time. On the other hand, the heart in many abnormal situations may actually be working against itself because of the asymmetric loading and thus be tolerating the stress poorly. Consequently, the efficiency of the heart and its impaired response to stress is intimately linked with the mechanics of ventricular contraction and relaxation as influenced by disproportionate loading and asymmetrical hypertrophy.

It is therefore apparent that these derangements will be associated with abnormal movements of the heart. One possible approach is the study in detail of the movements and shape-changes of the heart in normal and abnormal situations. Although there have been a number of attempts to determine the movements of the human and dog heart, the findings are very difficult to interpret and lack many of the necessary features for careful analysis. It is to be remembered that the contractile process probably takes place within twelve-hundredths of a second, making it necessary for any device used in measuring these phenomena to be exceedingly rapid in response and to offer no distortion to the contractile process. Most studies in the past have not been sufficiently sensitive for accurate representation and lack careful simultaneous timing. The detailed sequence of the heart movements and the mechanics associated with ventricular contraction and relaxation remain largely speculative. The statement of Sir William Harvey that the movements of the heart occur "in the twinkling of an eye, like a flash of lightning" and the statement of Fracastorius that "the motion of the heart was to be understood by God alone,"[8] still apply.

In view of the above-mentioned deficiencies in knowledge of cardiac mechanics, it is difficult to understand the general apathy today toward appropriate research. It seems imperative that more consideration and study be given these fundamental problems; in studying the mechanics of circulation, our attention should be directed back to Harvey.

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5. Mackenzie, Sir James: Diseases of the Heart (Fourth Edition). London, Oxford Univ. Press, 1925.
6. Smith, L. A., Fields, J., Kennamer, R., and Prinzmetal, R.: Studies on the mechanism of ventricular activity, III. Contraction of the ventricles in experimental bundle branch block. Am. Heart J. **44:** 231–237, 1952.
7. Massumi, R. A., Goldman, A., Rakita, L., Kuramoto, K., and Prinzmetal, M.: Studies on the mechanism of ventricular activity. Am. J. Med. **19:** 832–848, 1955.
8. Fracastorius, quoted by Sir William Harvey in De Motu Cordis.

CORRESPONDENCE

TO THE EDITOR:

I hope that the following *opinions* concerning the national meeting of the Federation may be . . . brought to the attention of those charged with arrangements for the meeting.

The present practice of presenting rapid-fire 10-minute papers to a large audience in a huge hall appears to violate reasonable precepts of both education and research. This practice mitigates against lucid and logical development of the work of the speaker because of the sheer rush to beat the clock. Perhaps others share the experience of usually being unable to follow even those minute-mad masterpieces which fall within their own investigative bailiwick.

It also appears evident that current practice tends to stifle audience participation during the discussion period. The program is eternally "behind schedule;" the audience is tremendous in number and large fractions of it move noisily in and out of the hall; there is no blackboard which can be written on so that general visibility is assured. In short, the atmosphere of national meetings appears to be conducive to authoritarian speed-ball pontifications of "new facts" rather than to an environment characterized by relaxed but critical exchange of question and argument.

It appears reasonable to assume that a cardinal purpose of a meeting of researchers is to promote clear presentation of data, methodology, and inferences as well as to afford opportunity for open, thoughtful, and critical argument. This purpose, I submit, is stifled by a beat-the-clock escapism which tends to help us evade the dual responsibilities of adequate presentation of data and of encouraging free discussion. Are not honest controversy, polemics, heated disputes the stuff of which further experimental refutation or confirmation is made?

An approach to a structural remedy may first be found in permitting twenty to thirty minutes for each presentation. This could be followed by two previously-invited discussors who may have five minutes each. The speaker could comment immediately after each discussor. Finally, general discussion would be encouraged for ten to twenty minutes with a chance for rebuttal or comment by the speaker again after each discussor.

If it is felt that an inadequate number of worthwhile papers could be presented in any one day, then it may be well to sectionalize the meetings into groups which gather concomitantly and separately. This would in any case acknowledge the fact that precious few investigators today are capable of grasping and contributing to research which transcends their limited sphere. It could also nullify the paradox of expecting a chairman who may be well-versed in, say, cardiopulmonary function to provoke discussion and critical analysis of papers dealing with viruses or nutrition.

The evening sectional meetings appear to have approached the aims cited here more closely than the general meetings during the day. But why have meetings going during the day and evening? That long lunch hours and free evenings are conducive to reflection, renewal of friendships, and interchange of ideas appears to be obvious.

It is apparent that the above suggestions are but one method of attempting to inject into our society the dignity and stature accompanying formal meetings which encourage leisurely presentation and examination of data, and critical argument. Should not our society take vigorous steps to achieve these ends?

R. Tarail, M.D.
Rosewall Park Memorial Institute
Buffalo, October 10, 1956

NOTICES

Call for Abstracts
National Meeting, May 5, 1957

The 14th Annual Meeting of the AMERICAN FEDERATION FOR CLINICAL RESEARCH will be held in the Steel Pier Theater, Atlantic City, New Jersey, on Sunday, May 5, 1957. There will be a general session from 9:00 a.m. to 5:00 p.m. and Subsection Meetings at 8:00 p.m. in the following fields:

Medical Education
Cardiovascular Disease
Renal Disease and Electrolyte Metabolism
Gastroenterology

All papers will be considered for the general session. Those not included in the general session program will still be considered for the Subsection Meetings listed above.

All abstracts should be sent to

Lawrence E. Hinkle, Jr., M.D.
P-200, The New York Hospital
525 East 68th Street
New York 21, New York

Rules Applying to Abstracts

1. Five copies must be submitted. Abstracts must be double-spaced and must not exceed 300 words.

2. No tables or graphs can be published.

3. Authors should refer to CLINICAL RESEARCH PROCEEDINGS, vol. I, p. 118 (September 1953) for pertinent suggestions on the form to be used in submitting research abstracts.

4. Each abstract must be accompanied by a covering letter giving the name, address, age and membership status of each of the authors and stating which author will present the paper. No one who has passed his forty-first birthday may present the paper.

5. One of the authors must be a member, or the paper must be introduced by a member.

6. Abstracts must be postmarked not later than February 25, 1957. Air mail should be used whenever delivery by regular mail cannot be accomplished within 36 hours.

Application for Membership

1. Young research workers are encouraged to apply for membership. It is unnecessary to await a member's invitation to join the AMERICAN FEDERATION FOR CLINICAL RESEARCH.

2. There is one requirement for regular membership: publication of a meritorious investigation in clinical medicine or allied sciences. This should not be a case report or an abstract.

3. An applicant must ask a member of the Federation who knows him to sign his application.

4. Interested individuals should write to Dr. William W. Stead, Secretary-Treasurer, AMERICAN FEDERATION FOR CLINICAL RESEARCH, V. A. Hospital, Minneapolis 17, Minnesota.

Members Lost to the National Office

It would be appreciated if each member would review the following list and notify Dr. William W. Stead (V. A. Hospital, Minneapolis 17, Minnesota) of the current address of anyone on the list with whom he is in contact:

	Former address
Dr. Ronald D. T. Cape	7 St. Augustine's Road, Edgbaston Birmingham 16, England
Dr. George L. Forbes, Jr.	232 Granada San Clemento, California
Dr. Goffredo G. Gensini	1265 Detroit Street Denver, Colorado,
Dr. Michele Gerundo	Guam Memorial Hospital Guam, M. I.
Dr. John F. Gillespie	2222 Eye Street, N. W. Washington, D. C.
Dr. Marvin M. Hirsch	Dept. of Preventive Medicine U. of Illinois College of Medicine 1857 West Polk Street Chicago 12, Illinois
Maj. Robert L. Johnson, USAF, MC	3750th Medical Group Sheppard AFB Wichita Falls, Texas
Dr. Truman G. Schnabel, Jr.	% Lars Werko Saint Eric's Hospital Stockholm, Sweden
Dr. Walter S. Wiggins	721 South Main Street Carrollton, Illinois
Dr. Irvin Zeavin	4145 Garfield Street Lincoln, Nebraska

Obituaries

The National Office has been notified of the death of the following members:

Dr. Jay A. Robinson, Trenton, New Jersey.

Dr. Joseph L. Lilienthal, Jr., Baltimore, Maryland, on November 19, 1955.

Publication by Other Societies

In order to broaden its usefulness to medical investigators, CLINICAL RESEARCH PROCEEDINGS will, if suitable arrangements can be made, publish advance research reports of societies other than the AMERICAN FEDERATION FOR CLINICAL RESEARCH. Advance reports of the *Western Society for Clinical Research* appear in this issue.

William W. Stead, M.D.

PROGRAM, EASTERN SECTION

American Federation for Clinical Research

Friday, Saturday, December 7, 8, 1956

Hurd Hall, Johns Hopkins Hospital, and Gordon Wilson Hall, University of Maryland Hospital, Baltimore, Maryland

Dr. Victor A. McKusick, Presiding

Presentations will be limited to ten minutes, with ten minutes for discussion

FRIDAY, DECEMBER 7

9:00 a.m.

1. **Renal Cortical Necrosis.**
   David P. Lauler and George E. Schreiner,* Washington. ***page 24***
2. **Kidney Function During Acute Tubular Necrosis: Clinical Studies and a Theory.**
   William H. Meroney and Milton E. Rubini, Washington. ***page 24***
3. **Physiologic Studies of Water Diuresis in the Nephrotic Syndrome.**
   Mackenzie Walser and Jack Orloff, Bethesda, Md. ***page 23***
4. **A Re-examination of Water Diuresis, Including the Role of the Neurohypophyseal Antidiuretic Hormone (ADH).**
   Abraham G. White, New York. ***page 22***
5. **The Graded Effect of Purified Vasopressin and Urinary Solute on the Excretion of Solute-free Water.**
   Henry N. Wagner, Jr., Douglas G. Davidson and Jack Orloff,* Bethesda, Md. ***page 23***
6. **Permeability and Tubular Reabsorption During Infusions of L-norepinephrine in Dogs.**
   Willoughby Lathem, Pittsburgh, Pa. ***page 25***
7. **Urinary Osmolar Concentration in the Hydropenic State as a Measure of Renal Tubular Function.**
   Martin N. Frank, Leonard S. Dreifus, Fred Rarrick* and Samuel Bellet,†* Philadelphia. ***page 24***
8. **The Mechanism of Hypochloremia in Chronic Respiratory Acidosis.**
   Franklin H. Epstein, William Branscome and Howard Levitin,** New Haven, Conn. ***page 17***
9. **Fluorescein Labeled Antiglobulin Test Applied to Leukocyte Immunology.**
   T. H. Danaher, G. J. Friou and S. C. Finch,* New Haven, Conn. ***page 9***
10. **Host Resistance to Microbial Infection and Intoxication Unrelated to Specific Immunity.**
    Edward W. Hook and Robert R. Wagner,* Baltimore, Md. ***page 22***

To be presented if time permits:

11. **Clinical Studies of Urinary Aldosterone with a New Method of Isolation and Determination.**
    Jacques Genest, Wojciech Nowaczynski, Erich Koiw* and Barna Vityé,** Montreal, Quebec, Canada. ***page 15***
12. **Application of the Fluorescent Antibody Technic to the Immunology of the Group A Streptococcus.**
    George J. Friou, New Haven, Conn. ***page 22***

12:30 p.m.

LUNCHEON

Great Hall, Welch Medical Library

Guest Speaker: Professor Bentley Glass

"The Genetic Effects of Atomic and Other Radiations"

AFTERNOON SESSION: *Dr. William W. Faloon, Presiding*

2:00 p.m.

13. **Pathology of Adult Myxedema: Report of Ten Autopsied Cases.**
    Robert C. Douglass and Samuel D. Jacobson,† Eloise, Mich. ***page 13***
14. **A Study of Red Blood Cell Survival in Hypo- and Hyperthyroidism.**
    *Gerald P. Rodnan and Wallace N. Jensen,** Pittsburgh, Pa. ***page 8***
15. **The Effect of Thyrotropin on the Serum Pattern of Thyroid Hormones.**
    Jerrold D. Hydowitz and Walter L. Arons,* Pittsburgh, Pa. ***page 12***
16. **Marked Depression of the Circulating Protein-bound Iodine Concentration in the Absence of Clinical Hypothyroidism During Testosterone Administration.**
    *H. G. Keitel and M. Sherer,** Bethesda, Md. ***page 13***

* By Invitation
† Senior Member
‡ Deceased

17. **Effectiveness of a Formula Diet for Weight Reduction of Obese Outpatients.**
Alvan R. Feinstein, Vincent P. Dole and Irving L. Schwartz,** New York **page 17**

18. **Studies of the Effects of a Plasma Lipid Mobilizing Factor in Man.**
Gladys M. Miller, Chris J. D. Zarafonetis,† William A. Steiger, Joseph Seifter,* David Baeder* and Ralph Myerson,* Philadelphia. **page 17**

19. **Clinical Studies on Norethandrolone: an Anabolic, Progestational Steroid.**
Jeanne A. Epstein, Lee Vosburgh, Georgia Reid* and Herbert S. Kupperman,†* New York. **page 16**

20. **The Effect of Dietary Protein and L-triiodothyronine on the Disappearance Rate of Iodinated Albumin.**
Frank L. Iber, Irvin C. Plough, William H. Meroney and Kenneth Fremont-Smith,* Washington. **page 18**

21. **Effect of Insulin on Glucose Uptake, Lactate Production and R. Q. in the Forearm of Man.**
Reubin Andres and Kenneth L. Zierler, Baltimore. **page 14**

22. **Effect of Potassium on the Resting Membrane Potential of Skeletal Muscle in the Intact Animal.**
John C. Harvey, Joseph L. Lilienthal, Jr.‡ and Kenneth L. Zierler, Baltimore. **page 18**

To be presented if time permits:

23. **The Effects of Reserpine in Hyperthyroidism.**
John J. Canary and Marcus Schaaf,* Washington. **page 12**

6:00 p.m.

COCKTAIL PARTY

Lord Baltimore Hotel

Courtesy of Wyeth Laboratories

SATURDAY, DECEMBER 8

9:00 a.m.

BUSINESS MEETING

Gordon Wilson Hall, University of Maryland Hospital

9:15 a.m.: Incoming Chairman Presiding

24. **The Effect of Histamine on Pepsin Secretion in the Human Stomach.**
E. Friedman, I. Poliner* and H. M. Spiro**, New Haven, Conn. (*Introduced by Victor A McKusick, M.D.*) **page 19**

25. **The Effect of Serotonin on Intestinal Motor Function in Man.**
Thomas R. Hendrix, Michael Atkinson*, James A. Clifton* and Franz J. Ingelfinger**, Boston. (*Introduced by Victor A. McKusick, M.D.*) **page 19**

26. **The Effect of Oral Protein and Glucose Feeding on Hepatic Vein Wedge Pressure.**
J. Leonard Brandt, Leonard Castleman and Herman D. Ruskin,†*, New York. **page 21**

27. **Clinical Significance of the Partial Pressure of Ammonia (PNH_3) in Patients with Ammonium Toxicity.**
John A. Jaquez, J. William Poppell*, Walter Lawrence, Jr.* and Kathleen E. Roberts,* New York. **page 20**

28. **The Clinical Significance of Alterations of Serum Glutamic Pyruvic (SGP) Transaminase in Hepatic Disease.**
Felix Wroblewski and John S. LaDue,† New York. **page 21**

29. **Observations on the Excretion of Formiminoglutamic Acid in Folic Acid Deficiency in Man.**
A. Leonard Luhby, New York. **page 8**

30. **Production of Antihuman PTC and Antihuman Proconvertin in Rabbits.**
Paul Didisheim and Jessica H. Lewis,* Pittsburgh, Pa. **page 9**

31. **The Coexistence of Patent Ductus Arteriosus (PDA) and Aortic Valvular Disease.**
Herbert Mark, Belle Jacobson and Dennison Young**, New York. **page 10**

32. **Evaluation of Mitral Commissurotomy by Combined Heart Catheterization.**
*Harry Goldberg and Janet Dickens**, Philadelphia. **page 10**

33. **The Treatment of Acute Barbiturate Intoxication with B-B-methylethyl-glutarimide (N P 13-Megimide).**
George E. Schreiner, Renato D. Kovach and Leonard B. Berman,* Washington. **page 26**

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Advance Reports Submitted to the Annual Meeting of the EASTERN SECTION of the American Federation for Clinical Research

Hurd Hall, Johns Hopkins Hospital and Gordon Wilson Hall, University of Maryland, Baltimore, Maryland · Friday, Saturday, December 7, 8, 1956

BLOOD

A Study of Red Blood Cell Survival in Hypo- and Hyper-Thyroidism

By *Gerald P. Rodman and Wallace N. Jensen.* Department of Medicine, University of Pittsburgh, Pittsburgh, Pa.

The survival of auto-transfused erythrocytes labelled *in vitro* with chromium^{51} ($Na_2Cr^{51}O_4$) has been investigated in a group of eight patients (7 female, 1 male) with well-documented hypothyroidism. Five of these patients were found to be anemic, having hematocrits of 31–33%. The erythrocytes were normal or slightly macrocytic in size, and marrow aspirates appeared normal or slightly hypocellular. In all cases, the mean life span of the patient's chromium-tagged red cells, when corrected for elution loss, was found to equal or exceed that of a group of normal women. The erythrocyte survival was estimated to be in excess of 150 days in 3 patients with profound myxedema. Repeat studies in these patients following correction of their thyroid dysfunction are in progress.

In a group of 7 patients with moderate to severe hyperthyroidism, none of whom was anemic, red cell survival appeared to fall within the normal range in all but one case. In this latter—a young boy with severe Graves' disease—the study indicated a markedly reduced red cell life span.

The data indicate that abnormal hemolysis plays no role in anemia of hypo-thyroidism and thus appear to confirm the previous impression (Rodnan, Ebaugh, and Fox) that there exists a relationship between red blood cell life span and metabolic rate.

Mediterranean Trait in a Negro Family

By *Harry Shecter and Abraham M. Frumin.* Department of Laboratories, Albert Einstein Medical Center, Southern Division, Philadelphia.

A negro female, born in South Carolina, one of 7 siblings, and 3 children were found to have a mild microcytic hypochromic anemia, reticulocytosis, with target cells, ovalocytes, and basophilic stippling in the peripheral blood smear. The hypotonic saline fragility test showed increased resistance. Serum bilirubin and fetal hemoglobin values were normal. Paper electrophoresis showed A hemoglobin. Serum iron values were normal. A Cr^{51} RBC survival of 23 days was obtained.

Another locus for Mediterranean trait in the American Negro has thus been demonstrated.

Observations on the Excretion of Formimino-Glutamic Acid in Folic Acid Deficiency in Man.

By *A. Leonard Luhby* (with the technical assistance of *H. P. Broquist, C. E. Rath, J. H. Burchenal and L. M. Murphy*). Department of Pediatrics, New York Medical College, New York City.

In folic acid deficiency induced in the rat by a deficient diet and succinylsulfathiazole, a derivative of glutamic acid is present in the urine which can be degraded by heat or alkali treatment to "free" glutamic acid as measured by the L-arabinosus growth assay. Silverman, Bakerman, Daft et al. have reported that the increased glutamic acid activity

appearing after heat or alkali treatment of the urine of deficient animals is directly proportional to the degree of granulocytopenia occurring in these rats, and hence presumably proportional to the degree of folic acid deficiency. This increased activity is absent in the normal rat and disappears after folic acid treatment of the PGA-deficient animal. Tabor et al.[7] have identified this glutamic acid derivative as formimino-L-glutamic acid.

We have found that a glutamic acid derivative, thus far microbiologically and chromatographically indistinguishable from formimino-L-glutamic acid, occurs in the urine of human subjects in certain folic acid deficiency states.

The urine of children with acute leukemia receiving folic acid antagonist therapy was found to possess an increased amount of glutamic acid activity following heat treatment of the urine. Paper chromatography of these urines showed the presence of a material which travelled at the same rate as synthetic formimino-L-glutamic acid. These characteristics disappeared upon withdrawal of the PGA antagonist. The urine of a patient with macrocytic anemia of pregnancy was also found to have the same microbiologic and chromatographic characteristics. The increased "glutamic acid" excretion in the heated urine became strikingly reduced following folic acid therapy. The urine of several patients with pernicious anemia in relapse was not found to possess significant amounts of glutamic acid in heated specimens. The urine of normal adults and children was uniformly negative, following heat treatment, for microbiologically detectable glutamic acid.

If isolation studies, which are in progress, prove this precursor compound to be formimino-L-glutamic acid, these observations will show for the first time that this biochemical abnormality exists in clinical folic acid deficiency states, and that PGA is concerned with formimino as well as methyl transfer in amino acid metabolism in man.

Fluorescein Labeled Antiglobulin Test Applied to Leukocyte Immunology

By *T. H. Danaher, G. J. Friou and S. C. Finch.* Department of Internal Medicine, Yale University School of Medicine, New Haven, and the U. S. V. A. Hospital, West Haven, Connecticut.

Fluorescein labeled antiglobulin was used in order to study the mechanism of leukocyte antibody reactions. Heterologous leukocyte antibody will produce agglutination, lysis and phagocytosis of human leukocytes. However, the detection and interpretation of similar human auto- and isoimmune reactions have been limited by lack of reliable technics. Spontaneous agglutination and rapid deterioration of leukocytes have seriously interfered with the evaluation of leukocyte antibody studies.

Antihuman globulin rabbit serum (Coombs serum) and antirabbit globulin chicken serum were labeled with fluorescein isocyanate according to the method of Coons. Each antiserum was absorbed with normal human erythrocytes. Washed human leukocyte smears were incubated with antihuman leukocyte rabbit serum followed by fluorescein labeled antirabbit globulin chicken serum. Upon inspection, strong leukocyte cytoplasmic fluorescence was noted with sparing of the nuclei. Unlabeled Coombs or normal rabbit serum followed by fluorescein tagged antirabbit globulin chicken serum showed moderate to minimal cytoplasmic fluorescence of the leukocytes. These studies indicate that heterologous leukocyte antibody localizes principally in the cytoplasm of normal human leukocytes. They also suggest that human globulin remains in or on washed normal human leukocyte cytoplasm.

The presence of human globulin in or on normal human leukocytes was demonstrated by other methods. Smears of washed (3× to 10×) human buffy coats were incubated with fluorescein tagged antihuman globulin rabbit serum. Strong leukocyte cytoplasmic fluorescence developed. The tagged antiserum was then repeatedly absorbed with washed human leukocytes. After each absorption the Coombs titer and globulin precipitation titer of the antiserum was checked against the capacity of this antiserum to stain leukocytes. It was found that disappearance of fluorescence of leukocytes incubated with this antiserum was well correlated with the loss of antiglobulin titer. Leukocytes from other mammalian species fluoresced with labeled rabbit antihuman globulin only after incubation with normal human serum. These studies suggest that human globulin is present in or avidly adheres to normal human leukocytes, and that normal human globulin becomes firmly adherent to many types of heterologous leukocytes.

Production of Antihuman PTC and Antihuman Proconvertin in Rabbits

By *Paul Didisheim and Jessica H. Lewis.* Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

The introduction of immunological technics into the field of blood coagulation holds promise of opening new approaches and techniques. Rabbits injected intramuscularly with a human serum fraction, "PTC," a citrate eluate of the barium sulfate adsorbate from human serum, develop an antibody to this fraction, which can be demonstrated by precipitin reactions and biological tests. The human PTC fraction injected contains 10 times the PTC concentration of whole plasma and a trace of proconvertin. The rabbit antiserum strongly inhibits human PTC and proconvertin but has no inhibitory effect on rabbit clotting factors. The anti-PTC and antiproconvertin activity could be separated

by differential absorption. When the rabbit $BaSO_4$-treated plasma was mixed with an equal volume of normal plasma, a heavy precipitate was formed. When this was removed by centrifugation no inhibitor activity could be demonstrated. After similar incubation of rabbit plasma with plasma from a patient with congenital PTC deficiency, a heavy precipitate was also formed, but after centrifugation, full anti-PTC activity could still be demonstrated. A similar mixture with hypoproconvertinemic plasma removed anti-PTC activity but left antiproconvertin activity. A PTC fraction prepared from human PTC-deficient plasma was injected into another series of rabbits. These rabbits developed antibody with antiproconvertin activity but no anti-PTC. Incubation of the $BaSO_4$ plasma from these rabbits with the normal PTC fraction produced a precipitate and lowered the proconvertin activity of the fraction but not the PTC activity.

CARDIORESPIRATORY SYSTEM

The Coexistence of Patent Ductus Arteriosus and Aortic Valvular Disease

By *Herbert Mark, Belle Jacobson and Dennison Young.* Medical Division, Montefiore Hospital, New York City.

Patent ductus arteriosus (PDA) as a rule exists as an isolated congenital anomaly in the noncyanotic patient. The coexistence of PDA and aortic valvular disease has rarely been reported in the American literature. The incidence given in the larger series (3 out of 412 patients by Gross and Longino) seems inordinately low.

This report is presented to indicate the criteria for diagnosis and the clinical complications in 7 patients out of 50 operated for PDA, in whom an aortic valvular lesion, presumably congenital aorta or subaortic stenosis, was present. This was confirmed by clinical findings, diagnostic carotid artery pulse tracings, and, in one case, by post-mortem examination.

Of the 7 patients, 5 were diagnosed preoperatively as having an associated aortic valvular lesion. In addition to the typical murmur of PDA with maximal transmission toward the left shoulder, the systolic and/or diastolic murmur assumed a different quality as they were traced to the aortic area. Two of these patients showed evidence of left ventricular enlargement out of proportion to the size of the shunt as estimated by clinical findings. In one patient the existence of aortic valvular involvement was not recognized until recovery from operation, when a persistent aortic systolic murmur with diagnostic carotid pulse tracing was found. One patient was not seen until one year after her PDA had been ligated. This had been followed by subacute bacterial endocarditis on the aortic valve causing rupture of a cusp with severe aortic insufficiency, and eventually leading to her death. Three of the 7 patients developed subacute bacterial endocarditis on the aortic valve. The presence of this associated congenital anomaly is, therefore, of more than academic interest.

It is concluded that a high level of suspicion for coexisting aortic valvular disease should be entertained if the auscultatory findings do not conform to the original description of Gibson given over fifty years ago.

Evaluation of Mitral Commissurotomy by Combined Heart Catheterization

By *Harry Goldberg and Janet Dickens.* Hahnemann Medical College and Hospital, Philadelphia.

Clinical changes following mitral commissurotomy often are difficult to evaluate. The need for an objective method in assessing the effectiveness of surgery is apparent. Right heart catheterization has not proven entirely satisfactory. Left heart catheterization provides a method for determination of the pressure gradient across the mitral valve. When combined with an estimation of the cardiac output (Direct Fick), the degree of stenosis may be determined more accurately than previously.

Fifty patients have been studied by the combined heart catheterization technique. Sixteen have been re-evaluated after mitral commissurotomy. Postoperative results, based upon an increase in mitral valve flow accompanied by a decrease in pressure gradient, were considered good in 10 patients. The functional valve area increased in all. Although the left atrial pressure decreased generally a good postoperative response was not always reflected by a fall in the pulmonary artery pressure. In one case the flow increased with no change in the pressure gradient, while in another both flow and gradient fell proportionately following surgery. A poor response was indicated in three patients showing little or no change in flow or gradient. In a case where the preoperative data was incomplete there was a small gradient with an adequate orifice size following surgery.

The degree of mitral obstruction was not necessarily reflected by the level of the pulmonary artery pressure. Pulmonary hypertension may not be the sole determining factor of the severity of stenosis or requirement of surgery when direct measurements of gradient and flow across the valve are known. These studies indicate that the estimation of the degree of mitral stenosis and response following com-

missurotomy cannot always be made by direct studies of the pulmonary circulation and cardiac output. The importance of simultaneous estimation of the pressure gradient and flow is emphasized.

The Value of Left Heart Catheterization in the Diagnosis and Treatment of Patients with Rheumatic Heart Disease

By *Joseph F. Uricchio, Janet Dickens, Lamberto Bentivoglio and Harry Goldberg*

Through the recent rapid increase in the use of left heart catheterization, considerable experience has been gained regarding its value in the diagnosis and treatment of rheumatic heart disease. The present report is based on the findings in 500 patients in whom this study was utilized. In 190 of these, combined right and left heart catheterizations were performed.

In effect, left heart catheterization extends the ability to assay circulatory dynamics by providing means for the determination of pressures in the left atrium, left ventricle and the root of the aorta. When this pressure data is recorded simultaneously with the estimations of cardiac output by the Direct Fick method, it becomes possible to obtain an accurate measurement of the magnitude of mitral and aortic stenosis.

From a diagnostic standpoint, the estimate of the severity of mitral and aortic obstruction is possible in patients with isolated or multiple lesions. This is particularly helpful when these lesions occur in the absence of characteristic clinical manifestations.

By defining the magnitude of mitral and aortic obstruction, left heart catheterization contributes to the decision regarding the necessity of surgical intervention. Furthermore, it serves as an accurate means of defining the results of such surgery. This has been emphasized by the recently revealed limitations of right heart catheterization in estimating the degree of mitral stenosis. It is now clear that pulmonary artery pressures and pulmonary hypertension may not be the sole determining factor of the severity of mitral stenosis or the requirements of surgery when the direct measurements of pressure gradients are known.

The Cardiac Response to Pulmonary Embolism

By *John J. Byrne and John M. Cahill.* Department of Surgery and the Surgical Service and Research Laboratory, Boston City Hospital, Boston.

The electrocardiographic pattern of pulmonary embolism was established in the rabbit using minute graphite particles. In general, it was similar to that seen in humans. The ST segment and T wave shifts, which probably indicate ventricular ischemia, were used for measuring the effects of various drugs and procedures on pulmonary embolism.

A standardized graphite dose produced ST changes in 9 of 10 controls and T wave changes in 8. One animal died within fifteen minutes. Vagectomy had little effect on the cardiac pattern.

Ether, nembutal, atropine, etamon, priscoline, subcutaneous and intravenous epinephrine, given 10 minutes prior to embolization, appeared to prevent these ST and T wave changes in varying degrees. Epinephrine given simultaneously with the embolization did not modify the EKG changes and had the highest death rate.

Since vagectomy does not affect the cardiac manifestations of pulmonary embolism, it is apparent that vago-cardiac reflexes are not present. The cardiac changes are secondary to the increased resistance of the pulmonary circuit which raises the intracavity pressures of the right ventricle producing an ischemia of the ventricular wall from mechanical pressure or diminution in coronary flow. However, these changes are prevented by drugs which reduce pulmonary arteriolar constriction. Thus, in addition to the mechanical blockage of the embolus, a pulmonary vasoconstriction is present which is due to a local vascular reflex.

The Influence of Plasmin upon Experimental Pulmonary Emboli and Pulmonary Infarcts

By *Paul Ruegsegger, Irwin Nydick, Eugene E. Cliffton and John S. LaDue*

The effects of plasmin, a fibrinolytic enzyme, were studied during different phases of the evolution of pulmonary infarcts in rabbits.

A method was devised whereby pulmonary infarction could be produced consistently following embolization. Plasmin was administered intravenously in varying dosages at different times following embolization and its influence upon the animals' course was studied.

Polyethylene catheters were threaded into the right heart of rabbits via the jugular vein. Preformed clots of different sizes were injected into these catheters in a group of 17 control rabbits and a group of 15 "treated" rabbits.

Sixteen of the 17 control rabbits revealed pulmonary infarction at autopsy, usually extensive in degree. Four of these rabbits had died within 24 hours after embolization from shock or acute right heart failure.

Plasmin was administered to the treated group in dose of 6 to 9 mg./Kg. body weight. All of the plasmin-treated rabbits survived. None of the 5 rabbits treated within 40 minutes after embolization showed infarction at autopsy. Of 6 rabbits treated within 4 to 24 hours, the lungs were normal in 3 and single small residual infarcts were seen in the other 3. Two animals treated after 40 hours showed somewhat larger areas of infarction. Two animals were treated promptly after two separate series of embolization. Both survived, one with clear lungs and the other with two small infarcts, whereas the control rabbit died promptly after the first embolization.

ENDOCRINES AND METABOLISM

The Rate of Degradation of Thyroxine in Patients with Thyroid Disease

By *Kenneth Sterling*. Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, New York.

The disappearance curve of plasma radioactivity after intravenous injection of I^{131} labeled L-thyroxine was employed to determine the rate of hormone degradation or removal. Studies were carried out before and after therapy in patients with thyroid disease.

In untreated subjects, thyroxine degradation was slower than normal in myxedema, and accelerated in thyrotoxicosis. Follow-up studies were carried out after appropriate therapy and attainment of the euthyroid state, as determined by clinical appearance, basal metabolic rate, and plasma protein-bound iodine concentration.

The initial abnormal disappearance curves had usually reverted to normal or nearly so when patients were restudied months to years after treatment.

In 5 myxedematous patients on prolonged replacement therapy, the degradation rate had returned to the normal range or slightly below. In contrast, no alteration in the disappearance curve occurred in subjects followed for 1 to 3 weeks after rapid treatment with triiodothyronine.

After treatment of thyrotoxicosis with radioiodine, surgery, or antithyroid drugs, the degradation rate became normal or approximately normal in 17 subjects. In one instance the rate remained markedly accelerated despite clinical euthyroidism. In two cases of post-radioiodine hypothyroidism, the rate was slower than normal, resembling the findings in spontaneously occurring myxedema.

The results suggest that the rate of removal of circulating thyroxine is a significant parameter of thyroid hormone metabolism.

The Effect of Thyrotropin on the Serum Pattern of Thyroid Hormones

By *Jerrold D. Hydovitz and Walter L. Arons*. Department of Medicine of the Hospital of the University of Pennsylvania, Philadelphia, and the Montefiore Hospital, Pittsburgh.

Radiopaperchromatographic studies have been carried out on the peripheral serum of 8 patients following the intramuscular administration of 10 to 20 units of thyroid stimulating hormone (TSH). All subjects had received therapeutic doses of radioactive iodine (I^{131}) 48 to 72 hours prior to the administration of the TSH. Chromatographic separation of thyroid hormones was achieved by the use of an ascending butanol-ammonia solvent system. The effect of thyrotropin on the serum patterns of thyroxine, triiodothyronine, monoiiodotyrosine and diiodotyrosine was investigated in 4 hyperthyroid and 4 euthyroid individuals. In both the euthyroid and the hyperthyroid groups the intramuscular administration of TSH was followed by a prompt increase (within 3 to 6 hours) of the thyroxine radioactivity in serum. In the 4 euthyroid subjects no change in radioactive triiodothyronine serum levels was noted except in one subject in whom an increase in triiodothyronine occurred approximately 30 hours after the administration of thyroid stimulating hormone. By contrast, 3 of the 4 hyperthyroid patients demonstrated small and transient rises in triiodothyronine levels within 2 to 6 hours after the thyrotropin had been administered. Two-dimensional chromatograms carried out before and after TSH administration according to the technique of Roche et al. have failed to demonstrate any additional iodine-containing substances in serum other than the four compounds enumerated above. These data would indicate that the administration of thyrotropin is followed by the rapid appearance of small amounts of triiodothyronine in the serum of hyperthyroid subjects while a similar response could not be demonstrated in the euthyroid patients.

The Effects of Reserpine in Hyperthyroidism

By *John J. Canary and Marcus Schaaf*. Department of Medicine, Georgetown University School of Medicine, and the Georgetown University Medical Division, District of Columbia General Hospital, Washington, D. C.

Because of the suggested relationship of the diencephalon and the sympathetic nervous system to hyperthyroidism, Reserpine, a diencephalic tranquilizing agent, was administered to 21 thyrotoxic subjects in an attempt to interrupt the disease at a possible site of origin. In euthyroidal subjects Reserpine has produced bradycardia and ptosis, increased the body weight and lowered the BMR.

Eleven patients received 0.75 to 4.0 mg. of oral Reserpine daily for 14 to 104 days. Symptoms disappeared in 8 moderately thyrotoxic subjects and improved in 3 severely toxic patients. The pulse slowed in all, and weight gain averaged 9 pounds. Tremor and skin and eye changes cleared in 8 patients and improved in all. The thyroid gland remained unchanged in size and consistency. Serial RAI and PBI determinations in 9 patients showed no significant changes.

Ten hospitalized patients with severe hyperthyroidism were given 7.5 to 15 mg. of intramuscular Reserpine daily for 1 to 16 days. Decrease in nervousness, pulse respiratory rate, sweating and tremor and improvement in eye signs were notice-

able within 4 to 6 hours. 5 mg. intramuscular doses of Reserpine frequently produced distressing side effects of weakness, dizziness, nasal congestion and headache, which delayed improvement. Serial RAI determinations were unchanged in 2 patients.

Despite clinical improvement, persistently elevated PBI and RAI determinations indicated that Reserpine had not interrupted the basic disease process. Clinical improvement possibly resulted from diminished activity of the sympathetic nervous system induced by Reserpine.

These studies suggest that Reserpine may mask the features of thyrotoxicosis and should never be the sole therapeutic agent in this disorder. Oral Reserpine deserves further trial as adjunctive therapy in thyrotoxicosis, and larger intramuscular doses appear valuable for the rapid control of symptoms of severe hyperthyroidism.

Pathology of Adult Myxedema: Report of Ten Autopsied Cases

By *Robert C. Douglass and Samuel D. Jacobson.* Wayne County General Hospital, Eloise, Michigan.

The literature on the pathology of adult myxedema, based on autopsied cases, is meager. Histochemical findings have been rarely described. Nine clinically-recognized cases of adult primary myxedema, studied by routine autopsy means plus histochemical methods, present features which are not well-known.

The thyroid gland was atrophied and replaced by fibrous tissue with occasional scattered groups of small follicles surrounded by lymphocytes, plasma cells and histiocytes. Epithelium showed degenerative changes with Hürthle cell and squamous metaplasia. The alterations may be primarily inflammatory in nature or those of secondary change to a primary degenerative process.

The skin exhibited hyperkeratosis, epidermal atrophy and the presence of mucoid substance in the upper dermis. The latter area was basophilic with hematoxylin, metachromatic with toluidine blue and slightly positive with mucicarmine.

Mucoid substance was present in the subepithelial stroma of the tongue and vocal cord.

Significant coronary arteriosclerosis was present in each case and cardiac hypertrophy in most. Many individual myocardial fibers were occupied by vacuolated areas which stained basophilic with hematoxylin and positive with PAS. This finding—basophilic degeneration of the myocardium—occurs in other cardiac disorders also.

Marked dilatation of the colon was present in 4 cases. In every case, numerous tissue mast cells were scattered throughout the muscularis propria and subserous connective tissue of the intestine. Areas of interstitial mucoid substance were often present where the mast cells were most abundant. Fibers of the muscularis were frequently swollen.

The relationship of mast cell proliferation and acid mucopolysaccharide deposition in primary myxedema is speculative. Asboe-Hansen believes that TSH excess may stimulate mast cell proliferation in the connective tissue which gives rise to mucopolysaccharide deposition. Mast cells and mucopolysaccharide may both represent response to the same connective tissue stimulus (Altshuler). In accord with either hypothesis are the findings presented. Similar alterations in thyroid gland, dermis, myocardium and intestine were not present in a single case of pituitary myxedema (panhypopituitarism), where TSH was lacking.

Marked Depression of the Circulating Protein-Bound Iodine Concentration in the Absence of Clinical Hypothyroidism during Testosterone Administration

By *H. G. Keitel and M. Sherer.* National Institute of Health, Bethesda, Maryland.

Hypercholesterolemia is frequently observed during testosterone administration; therefore the thyroidal radioiodine uptake and PBI were measured in subjects receiving testosterone to see whether a change in thyroid metabolism could account for the cholesterol findings. Twenty-five mg. of methyl testosterone were given orally 3 to 4 times a day for one month to 6 dwarfed prepubertal children, and 50 mg. of testosterone propionate were given intramuscularly 3 times a week for one month to 7 adults with carcinoma of the breast. The children gained weight (6–15%), had an increase in appetite, were more active and grew more rapidly, but no clinical changes were noted in the adults during the administration of testosterone. The control PBI and I^{131} thyroidal uptake were normal. Within two weeks, 12 of the 13 patients had a reduction in the PBI—in 9 instances to the hypothyroid range—and 8 of 11 had a reduction in the thyroidal I^{131} uptake during testosterone administration. The alkaline phosphatase concentration of plasma in the children remained unchanged or rose.

Production of Exophthalmosis in the Fundulus by Thyroid Substances

By *Herbert G. Langford.* Bermuda Biological Station, Bermuda, and the University of Mississippi School of Medicine.

The minnow, Fundulus heteroclitus, has been used previously for studies on the production of exophthalmos. In the present study the degree of exophthalmosis was determined by measuring daily the intercorneal distance with a vernier caliper by the method of Dobyns. A control group was com-

pared with groups given 0.1, 0.2, 0.5, and 1.0 mcg. triiodothyronine, 0.6 mg. desiccated thyroid, and 0.2 mg. TSH (Armour #317-52) daily for four days. The injections were given intraperitoneally. There was a significant development of exophthalmosis in the triiodothyronine and thyroid-treated groups compared to the controls ($p < 0.01$). No exophthalmosis developed in the TSH-treated animals, and an attempt to increase the dose resulted in 100% mortality.

As TSH has been shown repeatedly to cause exophthalmosis in fish and mammals, we do not place great significance upon its failure in this series. However, as the results with the thyroid preparation are unequivocal, we suggest that the present impression of the *exclusive* role of the pituitary in experimental and human exophthalmosis deserves searching scrutiny.

Oral Sulfonamide Therapy in Diabetes Mellitus

By *Charles R. Shuman and William L. Winter, Jr.* Department of Medicine, Temple University Hospital, Philadelphia.

The use of BZ-55 in 50 diabetic patients revealed a satisfactory response in blood glucose levels upon withholding insulin in 26 patients previously receiving the hormone, and maintained a good level of control in 9 untreated diabetics with persistent hyperglycemia and glycosuria.

Four patients were observed to require significantly lower insulin doses while receiving BZ-55 than could be achieved prior to its use under hospital conditions.

Eleven patients failed to show any response to treatment with the sulfonamides. Control of diabetes was rapidly lost upon cessation of insulin therapy or when the dose of insulin was lowered while receiving the drug.

Four patients with manifestations of insulin resistance responded with either a lowering of insulin dose or cessation of the hormone when given BZ-55 suggesting that increased requirements were due to enhanced insulinase activity.

In several instances, new diabetic patients responded symptomatically to control with BZ-55 in a manner similar to that observed with insulin-induced control of diabetes. This suggested potentiation of the suboptimal levels of endogenous insulin, probably by inhibition of insulinase, according to Mirsky.

Following surgery in mild or moderate diabetics, the increased insulin demand was rapidly lowered or obviated. This was not true in more severe cases.

Side effects observed in this series consisted of drug fever in 1 nondiabetic control subject; hypoglycemia in 3 instances, one of which was a diabetic and 2 controls; drug rash of mild degree in 2 diabetics which disappeared despite continuation of therapy. No renal, hepatic or hematological effects have been observed.

No conclusions concerning mechanisms of action can be drawn, but, in general, the successful results appear to develop in the maturity-onset type of diabetic in whom endogenous insulin may be expected to be found on the basis of previous reports.

Effect of Insulin on Glucose Uptake, Lactate Production and R.Q. in the Forearm of Man

By *Reubin Andres and Kenneth L. Zierler.* Departments of Environmental Medicine, and Medicine, the Johns Hopkins University and Hospital, Baltimore, Maryland (Supported by grants from ONR, NIH and Muscular Dystrophy Association).

Metabolic events in resting skeletal muscle (the forearm) were studied by measuring appropriate A-V concentration differences in 9 medical students in the basal state and, in addition, forearm blood flow in 6. Following a control period, insulin was injected into the brachial artery continuously for 26 minutes. The following points are of interest: 1. The dose of insulin, 0.0001 units/Kg. body weight/minute, is enough to produce profound local effects with no evident systemic effects, i.e. arterial glucose concentration does not fall. The injection increases the concentration of insulin in blood perfusing the forearm by only 3×10^{-4} units per ml. plasma on the average. 2. Glucose uptake is increased ten-fold, from control of 0.8 to 8.0 μM/min./100 ml. forearm at end of injection period. Oxygen consumption is unchanged. The increased uptake of glucose is more than twice that required to account for all the oxygen consumption of the forearm. 3. Yet the R.Q. of forearm tissues does not increase: pre-insulin = 0.67 ± 0.02, during insulin = 0.70 ± 0.03, post-insulin (1 hr.) = 0.65 ± 0.03. 4. Lactate production increases from a mean control value of 0.6 to a maximum of 1.1 μM/min./100 ml. forearm at the end of the injection period. During maximum glucose uptake lactate production accounts for less than 10% of the glucose. Insulin, then, does promote glucose uptake by muscle. However, the constant R.Q. and oxygen consumption suggest that mere availability of glucose within the cell does not determine its preferential use as an oxidative substrate nor does insulin per se induce its oxidation.

New Method for the Isolation and Determination of Urinary Aldosterone

By *Wojciech Nowaczynski, Erich Koiw and Jaques Genest.* Clinical Research Department, Hôtel-Dieu Hospital, Montreal, Canada.

Precise and accurate determinations of aldosterone are essential for a better understanding of the physiopathology of electrolytes regulation and of hypertension.

Our previous work has shown an increased urinary aldosterone excretion in patients with essential or malignant hypertension. Urinary extracts were purified, first in Zaffaroni's propylene glycol

toluene system, and second in the Bush C system. The extensive study of the aldosterone zone obtained in the latter chromatogram has revealed the presence of at least 5 different compounds in concentrations sufficient for the visual detection under the ultraviolet light.

This study has resulted in the elaboration of a new sensitive, specific and accurate method for the isolation and determination of urinary aldosterone in a high degree of purity.

Of the different procedures studied for the extraction and hydrolysis of urinary aldosterone, the most efficient ones appear to be: 1. the immediate extraction at pH 1 with chloroform and re-extraction following incubation at pH 4.5 with animal B-glucuronidase. 2. Continuous extraction at pH 1 for 35 hrs. in a Cohen-type extractor.

Aldosterone can be separated from other substances present in crude neutral urinary extracts by the successive use of the following procedures: 1. Silica Gel column: 2. Zaffaroni's propylene glycol/toluene system or preferably Nowaczynski and Koiw's ethylene glycol/toluene system; 3. Eberlein-Bongiovanni E_2B system, isooctane/butanol, water; 4. Bush B 5 system, benzene/55% aqueous methanol; 5. determination and identification by a) ultraviolet absorption at 239 millimicrons, b) blue tetrazolium reaction, c) absorption spectra in 100% phosphoric acid and in concentrated sulfuric acid.

Recovery of aldosterone added to an aliquot of urine was 83%.

The specificity and accuracy of our method is based on the Rf values for aldosterone in the three different chromatographic systems used, on the excellent agreement between the values obtained by ultra-violet and blue tetrazolium determinations, and on the similarity of the absorption curves of the chromogen spectra with those of pure aldosterone.

Data from pregnant women and from patients with anxiety states and other diseases have been obtained.

Clinical Studies of Urinary Aldosterone with a New Method of Isolation and Determination

By *Jacques Genest, Wojciech Nowaczynski, Erich Koiw and Barna Vityé.* Clinical Research Department, Hôtel-Dieu Hospital, Montreal, Canada.

Studies on a purified aldosterone fraction obtained after two successive chromatographic separations of crude neutral extracts of urine (Zaffaroni's propylene glycol/toluene and Bush C systems) have resulted in the isolation of aldosterone and of 4 other substances (11-dehydro aldosterone, Compounds III, IV and V). This study has permitted the elaboration of a new method of purification of aldosterone in biological fluids. The specificity and accuracy of this method is based on the separation of aldosterone in a very high degree of purity from other substances present in urinary extracts.

Seven different urinary aliquots from normal pregnant women in their 7th to 9th month of pregnancy were studied for their aldosterone content. These urines were extracted continuously at pH 1 for 24 hours. The following values were obtained: 105, 108, 69, 59, 57, 80 and 20 mcg. per day. In each case, the agreement between the values obtained by ultraviolet absorption at 240 μ and by the blue tetrazolium reaction was between 95 to 100%. With the exception of the last case, where insufficient material was available, the chromogen spectra in concentrated sulfuric acid and in 100% phosphoric acid were entirely similar to those of pure aldosterone.

Three cases on a high potassium intake (180 milliequivalents in addition to the usual diet) were studied by this method. In these cases, the Eberlein-Bongiovanni E_2B chromatographic separation was done following the Bush system. The findings of a three-fold increase in aldosterone during the period of high potassium intake confirms the work of Laragh, Liddle, Luetscher and their co-workers. But the most striking differences are the 30 to 100-fold increase in Compound III and in the appearance of a new substance much more polar than aldosterone during the period of high potassium intake. Compound III has shown in adrenalectomized rats sodium-diuretic properties in dosages varying between 2 and 5 mcg.

Data were obtained in normal subjects, in essential hypertension, in anxiety states and in other diseases.

Characterization of C_{21} Steroids in the Urine of Normal Children

By *Hortense M. Gandy.* Department of Medicine, Strong Memorial Hospital, University of Rochester School of Medicine, Rochester, New York. (Aided by a U. S. Public Health Service Fellowship.)

This study was undertaken in order to define more closely C_{21} steroid synthesis in normal children. Total reducing urinary corticoids were determined in 25 normal children ranging in age from 3 to 11 years. Total urinary corticoid excretion ranged from 1.2 mg./24 hours in the youngest subject to 4.8 mg./24 hours in the oldest subject. Corticoid excretion is apparently a function of increasing somatic growth.

The total urinary excretion pattern was subsequently studied quantitatively and qualitatively in 4 subjects as follows: 1. Glucuronidase hydrolysis followed by ether extraction; 2. Determination of total α ketols of the neutral extracts with blue tetrazolium; and 3. Separation of the individual α ketolic steroids by paper chromatography. The quantitative α ketol pattern as $C_{21}O_3$, $C_{21}O_4$ and $C_{21}O_5$ in these subjects is not unlike that of adults.

The α ketolic components of the $C_{21}O_5$ fraction have been further characterized on paper for polarity, ultra violet absorption, the presence of α ketols, alpha-beta unsaturation, the presence of ketones,

hydroxylation and glycerol configuration. Absorption maxima in methanol and sulfuric acid chromogens were also studied. α ketol urinary metabolites identified are tetrahydro F, tetrahydro E, and tetrahydro B. Three other compounds have been isolated but have not been completely identified. One of these compounds has a polarity and an absorption at 240 μ in methanol like compound F; however, the sulfuric acid chromogen is not characteristic of compound F. Alpha ketolic metabolites in the $C_{21}O_4$ and $C_{21}O_5$ are now under study.

The qualitative and quantitative α ketol excretion pattern of normal children has been compared to that of children with nephrosis and the adrenogenital syndrome in the same age range and these results were studied.

Clinical Studies on Norethandrolone: an Anabolic, Progestational Steroid

By *Jeanna A. Epstein, Lee Vosburgh, Georgia Reid and Herbert S. Kupperman.* Departments of Therapeutics and Obstetrics and Gynecology, New York University-Bellevue Medical Center, and the Obstetrical and Gynecological Service (Third Division), Bellevue Hospital, New York City.

In the search for an anabolic agent which might be effective without manifesting androgenicity—as does that most potent of all anabolic steroids, testosterone—a new series of compounds have been made available. One such recently developed synthetic steroid is 19-nor-17-ethyltestosterone (Norethandrolone) which has been reported to have anabolic activity with only minimal androgenicity. Structurally, Norethandrolone, a 21-carbon compound, is closely related to progesterone. Laboratory investigations on the pharmacologic properties of Norethandrolone, using the parabiotic rat technic, have been reported elsewhere and indicate that this substance is one with anabolic potential associated with minimal androgenicity and effective pituitary gonadotrophin inhibiting properties. The present report deals with the clinical responses in regard to anabolic, pituitary inhibiting, and progestational potential of Norethandrolone in a divergent group of patients. These patients were of both sexes, ranging in age from 2 to 58 years, and were underweight normals who had previously been unable to gain weight or had evidence of gonadal or pituitary failure. The anabolic activity of the drug was measured by edema-free weight increase. At a dose level of 0.5 mg./Kg. body weight, androgenicity was frequently evident in pre-pubertal or hypogonadal males. On 20 to 30 mg. of the drug daily, for an average of 8.6 weeks of treatment, 29 of the cases gained an average of 5.39 pounds, 8 lost an average of 3.73 lbs. and one case showed no weight change. Norethandrolone induced menstrual irregularities in underweight females with regular menses. This was due to the drug's progestational activity rather than its androgenicity. We have obtained data showing the effect of Norethandrolone on basal body temperature curves, endometrial biopsies, and menstrual responses in women with various gonadal defects. Gonadotrophin inhibiting activity was accomplished with 20 mg. of Norethandrolone per day in two males with Klinefelter's syndrome and with 40 mg. per day in a menopausal female. Thirty mg. of Norethandrolone failed to depress FSH in this patient as well as in two other menopausal females.

Corticosteroid and 17-Ketosteroid Excretion in Paranoid Schizophrenia

By *Aniela S. Zygmuntowicz, Charles G. Colburn, Edwin J. Fredenburgh and Charles A. Pinderhughes.* Research Laboratory, V. A. Hospital, Bedford, Massachusetts.

Reported investigations into adrenal activity and responsiveness to stress in psychotic individuals frequently conflict in their conclusions concerning these functions. The discrepancies have undoubtedly resulted from a tendency to make limited observations in group studies. This report deals with a three-month study of a single patient suffering from paranoid schizophrenia.

Ninety-five consecutive 24-hour urines were obtained from a 34-year-old male paraplegic by means of an external catheter draining directly into the sample bottle. Collections were considered accurate and complete, with few exceptions. All were analyzed quantitatively for corticosteroids and 17-ketosteroids, with qualitative fractionation of selected specimens of the latter.

Clinically, the subject was overtly psychotic for the first 45 days. He underwent no therapy throughout the study, although he was interviewed briefly by a psychiatrist daily for evaluation of his mental state. Stimuli, deliberately planned and unplanned during the psychotic period, occurred on days 16, 20 and 33. The latter resulted in extremely agitated behavior until day 45. During the following weeks, which constituted a clinical recovery period, day 62 was marked by the withdrawal of the psychiatrist from the study for reasons of illness and days 80–86 by the absence of the ward physician while plans for discharge were being considered.

For the first 15 days, 17-ketosteroids were in the normal range (10–20 mg.) and corticosteroids at the low to low normal levels (normal 0.12–0.38 mg.). During the next 30 days, 17-ketosteroids showed exaggerated swings between 6.8 and 15.9 mg., while corticosteroids were essentially unchanged. After day 45, 17-ketosteroids fell far below normal and remained depressed until discharge. Corticosteroids from day 45 to 65 were well within normal limits. From day 65 to 90 excretion was elevated, fluctuating between 0.4 and 0.9 mg. It returned to normal during the last 5 hospital days.

Chromatographic fractionations of 17-ketosteroids showed a quite constant qualitative pattern throughout, despite quantitative changes in both 17-ketosteroids and corticosteroids. A notable feature of those patterns was a depression of the peaks in the areas of elution of corticosteroid metabolites. Failure to demonstrate any measurable qualitative change in these metabolites suggests a defect in the system of adrenal cortical production and/or utilization. The ability of the adrenals to respond to psychic stress apparently was impaired during the acute phase of the patient's illness, though he was physiologically capable of response during the recovery period.

Studies of the Effects of a Plasma Lipid Mobilizing Factor in Man

By *Gladys M. Miller, Chris J. Zarafonetis, William A. Steiger, Joseph Seifter, David Baeder and Ralph Myerson*

Steiger et al and Zarafonetis et al. recently reported observation of the effects in man of a plsama dialysate which contains a naturally occurring lipid mobilizing factor (LMF). Marked increases in plasma total cholesterol, total fatty acids, and lipid phosphorus resulted from a single intravenous injection of LMF into each of 25 fasting subjects. In order to characterize further the effects of LMF, studies have been extended to: (1) additional subjects, both fasting and non-fasting; (2) metabolic studies in 4 subjects and (3) initial tests in man of the purified active principles of LMF.

In the balance studies, patients were maintained for 4 weeks on a liquid formula diet which provided a daily average of 2230 calories, with an average of 381 Gm. carbohydrate, 166 Gm. protein, and 4.8 Gm. fat. The first and fourth weeks were control periods, while during the second and third weeks, daily intravenous injections of LMF were given in doses increasing from 1.0 to 10 mg./Kg. Injection of LMF was followed by a significant rise in plasma cholesterol, total fatty acids, and phospholipids in each subject. These remained elevated for as long as 1 to 2 weeks after the injections of LMF were discontinued. Urinary excretion of cholesterol increased approximately four-fold following administration of LMF, while both fecal and urinary nitrogen excretion decreased, as did the fecal fatty acids and lipid phosphorus. Excretion of cholesterol (Liebman-Burchard) in the feces increased two- to three-fold over control values.

Finally, in fasting subjects receiving as little as 1.2 mcg. of a purified crystalline product of LMF intravenously, there resulted a two- to three-fold rise in plasma lipids. The effectiveness of this minute quantity suggests the probable hormonal nature of this material.

The Mechanism of Hypochloremia in Chronic Respiratory Acidosis

By *Franklin H. Epstein, William Branscome and Howard Levitin.* Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Acute studies of CO_2 inhalation in man and animals have heretofore failed to demonstrate losses of chloride in the urine in excess of sodium. It has therefore been suggested by some that the characteristic hypochloremia of chronic respiratory acidosis might arise from transfer of chloride to an intracellular position or from dilution of the extracellular fluid with sodium bicarbonate.

Sixteen rats weighing 300–400 Gm. were placed in metabolism cages in an atmosphere containing 7½ per cent CO_2 in air or 12 per cent CO_2 in air, for 1 to 4 days, and sacrificed at the conclusion of the experiment. Breathing CO_2 produced an elevation in serum bicarbonate and a reciprocal depression of serum chloride of about 10 mEq./L. with 7½% CO_2 and 20 mEq./L. with 12% CO_2. These alterations were as marked at the end of one day as after three days. Renal losses of Cl^- and K^+ were increased on the first day of hypercapnia to produce a negative balance of these ions at the end of 24 hours, after which no further net loss occurred. A negative sodium balance for the first day, as well as larger negative balances of other ions, were observed in rats placed in 12% CO_2 but not in 7½% CO_2; this was complicated by diminished appetite and decreased food intake in the former rats, but not in those exposed to the lower concentration of CO_2. Ammonia excretion increased on exposure to CO_2 and returned promptly to normal when the rats were given room air to breathe.

The data suggest that renal compensation, characterized by excretion of Cl^- in association with K^+ and NH_4^+, plays an important role in adjustments to chronic respiratory acidosis in the rat.

Effectiveness of a Formula Diet for Weight Reduction of Obese Out-Patients

By *Alvan R. Feinstein, Vincent P. Dole and Irving L. Schwartz.* Hospital of the Rockefeller Institute, New York City.

One hundred six obese out-patients of average weight 224 lbs. were instructed in the use of a 900-calorie daily diet which permitted no intake of natural foods and in which the sole source of nutrients was a formula mixture, composed of evaporated milk, dextrose, corn oil and water. The mixture was prepared by the patients at home and taken in 5 or 6 daily feedings, supplemented by a multi-vitamin capsule. Noncaloric liquids were allowed ad lib. No adjunct medications for weight reduction were used.

The patients were followed at weekly or bi-weekly intervals in clinic.

Fifteen per cent of the patients were able to follow the diet scrupulously for over 11 weeks, 7% over 21 weeks. Although 56% of the patients began to take food within the first two weeks, almost half of these were able to control its intake as a modest supplement to the basic daily ration of formula mixture, and continued dieting, with progressive loss in weight. For those who followed the diet faithfully, the weight loss was 12–20 pounds in the first month and 1–3 pounds per week at a steady rate thereafter.

Data reported with other reduction regimens indicate that 12–29% of patients starting various diet procedures were able to lose 20 pounds or more, and 2–10% lost over 40 pounds. With the formula diet regimen, 53% of the patients lost over 20 pounds, 28% lost over 40 pounds, and 4 patients lost over 100 pounds each. No significant clinical or laboratory evidence of nutritional deficiency was encountered in any of the patients. In those who attained normal weight, the return to natural foods was made without digestive difficulty although constant dietary vigilance was required to avoid regaining lost weight.

The major disadvantage of the diet and the most frequent cause of its abandonment was monotony. Although optimal clinical supervision made significant contributions to the success of the regimen, certain intrinsic features of the diet played important roles. Chief among these were simplicity and inflexibility. It was evident that many obese patients preferred to abstain from natural food altogether than to cope with the complexities of calorie-counting in making selections among diverse foods, or to endure the frustration of limiting intake of the natural foods allowed in conventional diets.

The composition of the particular formula mixture used in this study is not considered essential for this type of diet, and mixtures of different compositions, equally calorically restricted, have shown similar results in preliminary studies on patients in the hospital and at home.

Effect of Potassium on the Resting Membrane Potential of Skeletal Muscle in the Intact Animal

By *John C. Harvey, Joseph L. Lilienthal, Jr. and Kenneth L. Zierler.* Departments of Environmental Medicine and Medicine, the Johns Hopkins University and Hospital, Baltimore, Maryland. (Aided by a contract with O.N.R.)

Resting membrane potentials (E_r) were measured with KCl filled glass microelectrodes introduced into single muscle fibers of the gastrocnemius of the intact rat. In 500 measurements in 49 rats E_r was 80.2 ± 7 mV. In 25 of these rats serum K^+ was measured and E_r was found to vary inversely with serum K^+. E_r was maintained for one-half to two hours in the impalements. Local ionic concentrations were altered without disturbing blood circulation by infusion of electrolyte solutions at the bifurcation of the aorta with a catheter inserted into the contralateral femoral artery. Concentrations of serum K^+ and Na^+ were measured in jugular venous blood and were taken as an index of the extracellular environment of the gastrocnemius. Intra-arterial infusion of 3 M NaCl (0.8 ml. in 10 min.) did not disturb E_r. Intra-arterial infusion of 3 M KCl (0.8 ml. in 10 min.) caused a prompt fall in E_r by 15–20 mV. This occurred before there was a detectable rise in jugular venous K^+ concentration.

The Effect of Dietary Protein and L-Triiodothyronine on the Disappearance Rate of Iodinated Albumin

By *Frank L. Iber, Irvin C. Plough, William H. Meroney and Kenneth Fremont-Smith.* Walter Reed Army Institute of Research, Washington, D. C.

The disappearance rate of human iodinated serum albumin (HISA) from the body was accelerated by feeding a high protein diet or by the administration of triiodothyronine (T_3). Both control and experimental observations were made following a single HISA injection. Five subjects in good nutritional status were studied on both low and high protein intake (0.5 Gm. and 3.0 Gm. protein/Kg. respectively). Generally the rate of disappearance of the label from the body was increased by changing from low to high protein intake; the rate was decreased when the change was in the opposite direction. The largest increased breakdown with change in the diet was 1.8 Gm. albumin/day. All subjects were in slight positive nitrogen balance during the high protein intake. Four subjects were studied before and after T_3 administration. T_3 was administered in a dose of 1.0 mg./day for 4 or 7 days. A profound negative nitrogen balance followed this administration and in each subject there was increased rate of disappearance of the iodine label from the body. The changes in turnover indicate that the serum albumin participates in acute changes in nitrogen metabolism.

GASTROINTESTINAL SYSTEM

The Effect of Histamine on Pepsin Secretion in the Human Stomach

By *E. Friedman, I. Poliner and H. M. Spiro.* Yale University School of Medicine, New Haven, Connecticut.

Since the gastric phase of gastric secretion is mediated by histamine or a closely related substance, whether histamine stimulates or merely "washes out" pepsin from the gastric glands is of more than theoretical interest. Discrepancies in previously recorded studies mainly carried out in animals exist partially because such studies have been limited to pepsin concentration, not *total* pepsin output, and partially because "permissive" factors from the central nervous system may provide a more complex background for gastric secretion in man than in animals.

This problem has been re-evaluated in human subjects, using the Anson-Mirsky hemoglobin method for measuring pepsin. Subjects who represented high, normal, and low levels of gastric secretion was given spaced successive doses of histamine over a 2 to 3 hour period. Histamine was given as closely as every 30 minutes; and in varying dosage from .5 mg. to 1 mg. of histamine base. Accumulation of pepsinogen in gland crypts could not reasonably play a role, since an augmented gastric output was maintained. In all cases, the total pepsin output remained at a level as high as that following the first injection of histamine throughout the course of each test. Pepsin concentration dropped slightly in patients with initial hypersecretion whose gastric juice volume rose enormously; but in subjects with an initial low or moderate gastric juice volume, the pepsin concentration and total output were well maintained at a higher level.

The Effect of Serotonin on Intestinal Motor Function in Man

By *Thomas R. Hendrix, Michael Atkinson, James A. Clifton and Franz J. Ingelfinger*

The argentaffin cells of the alimentary tract are believed to produce serotonin (5-hydroxytryptamine) which in vitro increases smooth muscle tone. Since diarrhea may be associated with carcinoid tumors producing serotonin, the intestinal effect of this substance was investigated.

Small intestinal motility was studied by the balloon kymograph method in normal subjects.

In 28 of 33 studies intravenous serotonin changed intestinal motor activity. In 24 intestinal tone abruptly rose 10–60 sec. after injection, and this "spasm" persisted for 1–8 min., after which tone and motor activity were depressed. In 4 studies serotonin injection was followed by a 1–3 min. period of depressed tonic and phasic activity. The usual threshold dose was 0.5–2.5 mg., but 4 individuals were insensitive to doses up to 3.0 mg. The subjective effects of intravenous serotonin (tingling, warmth and dyspnea) preceded the intestinal response and subsided before the intestinal manifestations disappeared. Abdominal cramps and diarrhea were not induced.

Serotonin in doses of 4–30 mg. instilled into the bowel next to the recording balloon did not alter motility in 12 subjects.

As it has been shown by others that intravenous serotonin can release histamine, intravenous histamine was given, but intestinal motility was not altered. Also, antihistaminic drugs did not inhibit the serotonin response; in fact potentiation, sometimes striking, occurred. Potentiation of the serotonin response by these agents may be ascribed to their inhibition of monoamine oxidase, an important enzyme in the degradation of serotonin.

To determine the site of serotonin action the effect of hexamethonium and methantheline on the intestinal response was studied. Ganglionic blockade did not alter the intestinal effect of serotonin, but the anticholinergic agent completely blocked this response. These observations suggest that the intestinal response to serotonin is mediated by the post-ganglionic parasympathetic system.

Therapeutic Appraisal Employing the Double-Blind Control Technic of a New Intestinal Anticholinergic Agent, JB-340 (Cantil®) in Various Types of Obstinate Diarrhea

By *Martin S. Kleckner, Jr.* Department of Medicine, Yale University School of Medicine and Hartford Hospital, Hartford, Connecticut.

Preliminary trial with JB-340 (Cantil) N-methyl-3-piperidyl-diphenylglycolate methobromide, a new synthetic post-ganglionic parasympathicolytic agent, disclosed unusually effective control of various obstinate diarrheas and that the activity of the drug was confined principally to the lower gastro-intestinal tract, corroborating earlier physiological studies. In addition, this agent has been shown to be active spasmolytically by roentgenographic motility, balloon-kymographic, proctoscopic and gastric secretory studies.

The therapeutic oral dosage was 25 mg. administered q.i.d., from which side effects were practically nil. The conditions studied in 27 patients were severe irritable bowel with diarrhea, regional enteritis, chronic ulcerative colitis, bacillary dysentery, gastro-jejunocolic fistula, and protracted diarrhea following ileostomy, colostomy, and massive resection of the small intestine.

Subjective and objective results with JB-340 therapy after a four-month period were so impressive in these conditions after no improvement by other well-known anticholinergic agents and a placebo that JB-340 was restudied, this time employing a double-blind control technique.

JB-340, 25 mg., atropine sulfate, grain 1/100, and a lactose placebo administered independently q.i.d. at four-week intervals were the pharmaceutical agents employed in the succeeding controlled investigation. This reconfirmed the initial impression that obstinate diarrhea associated, in particular, with the irritable bowel syndrome, regional enteritis, chronic ulcerative colitis, infectious diarrheas, and following small or large intestinal resection or enterostomy were controlled more effectively by JB-340 than by atropine or a placebo. Similarly, JB-340 was also found fluoroscopically to decrease intestinal motility in these conditions more consistently than the other agents.

Treatment of Hepatic Coma by Hemodialysis

By *John E. Kiley, Joseph C. Pender, Harold F. Welch and C. Stuart Welch.* Departments of Medicine and Surgery, Albany Medical College, Albany Hospital and the V. A. Hospital, Albany, New York (Aided by a grant from the U. S. Public Health Service).

Four patients with cirrhosis of the liver in coma with elevated blood ammonia levels were treated by hemodialysis using a Kolff-type artificial kidney. Ammonia levels were measured by a modified Conway technic. The quantity of ammonia removed was measured approximately by multiplying the rate of the flow of blood through the artificial kidney by the change in ammonia concentration in the blood during passage through the dialyzer.

All patients had severe liver disease and were completely unresponsive following gastrointestinal hemorrhage.

A 57-year-old man was treated for 4 hours. Forty-one liters of blood were dialyzed, approximately 75 mg. of ammonia nitrogen were removed, the arterial concentration of blood ammonia nitrogen changed from 277 to 138 mcg. percentage during dialysis, and the patient became alert the day following dialysis.

A 67-year-old man was treated for 6 hours. Thirty-six L. of blood were dialyzed, approximately 30 mg. of ammonia nitrogen were removed, the arterial concentration of blood ammonia nitrogen changed from 197 to 74 mcg. percentage during dialysis, and the patient became responsive to questions on the second day after dialysis.

A 43-year-old woman with ammonia intoxication, moderate uremia, and marked hypokalemia was treated for 6 hours. Forty-four liters of blood were dialyzed, approximately 75 mg. of ammonia nitrogen were removed, the arterial concentration of blood ammonia nitrogen changed from 150 to 123 mcg. percentage, and the patient became responsive to questions on the second day after dialysis.

Treatment of a 49-year-old man was terminated by technical difficulties after 2 hours. Eleven L. of blood were dialyzed, approximately 8 mg. of ammonia nitrogen were removed, the arterial concentration of blood ammonia nitrogen changed from 138 to 151 mcg. percentage during dialysis, and the patient remained in coma and died on the second day after dialysis.

These experiences indicate that it is possible to submit these patients to dialysis and that blood ammonia nitrogen can be removed by this technic.

Clinical Significance of the Partial Pressure of Ammonia (PNH_3) in Patients with Ammonium Toxicity

By *John A. Jaquez, J. William Poppell, Walter Lawrence, Jr. and Kathleen E. Roberts.* Departments of Chemotherapy and Medicine, Memorial Center, and the Andre and Bella Meyer Physiology Laboratories of the Sloan-Kettering Institute, New York City.

The correlation between the signs and symptoms displayed by patients in hepatic coma and the blood level of ammonium is generally considered to be poor. It has been noted, however, that patients with an elevated blood ammonium who have a superimposed respiratory alkalosis and/or an elevation of temperature show more clinical deterioration.

The ammonium in the blood is present in two forms in the NH_3-NH_4+ buffer system: as free ammonia (NH_3) and as the ammonium ion (NH_4). In this system an elevation of pH or temperature increases the ammonia (NH_3) fraction. This suggests that the significant factor in ammonium toxicity is the non-ionized ammonia, the concentration of which may be expressed in terms of the partial pressure of ammonia (PNH_3).

The plasma PNH_3 at various pH and temperature changes have been calculated and the PNH_3 correlated with the physical status of patients with an elevated blood ammonium. The results of these studies show a considerable overlap of the total blood ammonium values in the coma and noncomatose groups of patients. However, there was far less overlap of the PNH_3 values in the two groups of patients, and higher PNH_3 values were more frequently associated with coma.

It is concluded from these studies that the PNH_3 of the plasma, or some factor closely correlated with the PNH_3, is a more significant factor in precipitating toxicity than is the total blood ammonium.

The Effect of Oral Protein and Glucose Feeding on Hepatic Vein Wedge Pressure

By *J. Leonard Brandt, Leonard Castleman and Herman D. Ruskin.* Department of Medicine, State University of New York College of Medicine, New York City

During studies of the effects of oral protein and glucose feeding on splanchnic metabolism, it was noted that occasionally protein feeding was accompanied by a striking rise in hepatic vein wedge pressure (HVWP). Using standard technic for catheter wedging of the hepatic venule (Myers), a systematic study was therefore undertaken to determine what type of feedings were associated with a change in HVWP.

Fasting HVWP for 16 normal subjects averaged 87 mm. H_2O. For 19 cirrhotic subjects, fasting HVWP averaged 314 mm. H_2O and ranged as high as 458 mm. H_2O. Of 9 normal subjects given oral glucose (100 Gm. glucose in 500 cc. water), the average maximum change in HVWP was +18 mm. H_2O (one subject showed a fall of 55 mm. H_2O following the glucose feeding). Four cirrhotic subjects given the glucose feeding showed a mean rise of only 21 mm. H_2O.

For the subjects fed a protein meal (lean chopped beef containing 60 Gm. protein in 500 cc. volume) there was a higher mean rise in HVWP for both normals and cirrhotics as compared to glucose feeding. For the normals this averaged 30 mm. H_2O rise and for the cirrhotics 58 mm. H_2O rise. While comparison of the mean change in HVWP reveals no remarkable differences between normals and cirrhotics as a result of protein when compared to glucose feeding, there were 6 of 11 cirrhotics who showed rises in HVWP over 40 mm. H_2O after a protein meal. Two of these cirrhotics had rises in HVWP well over 150 mm. H_2O as a result of the protein feeding. One normal subject fed protein had a rise of 46 mm. H_2O in HVWP as a result of protein feeding.

While high protein feedings to decompensated cirrhotics are not uniformly followed by hepatic coma, the present study likewise indicates the inconsistent but striking effect protein feeding might have on portal vein pressure (as reflected in HVWP). These latter findings might explain the clinical observation that an occasional cirrhotic has a first major bleeding episode after being placed on a high protein dietary regimen.

The Clinical Significance of Alterations of Serum Glutamic Pyruvic (SGP) Transaminase in Hepatic Disease

By *Felix Wróblewski and John S. LaDue*

The distribution of glutamic-oxaloacetic transaminase and of glutamic pyruvic transaminase activity in homogenates of human tissues are different, the latter being most active in liver tissue. Both enzymes in the serum may be measured chromatographically, spectrophotometrically and colorimetrically. The activity, determined spectrophotometrically, of serum glutamic-oxaloacetic transaminase (SGO-T) in normal adult individuals has a mean value of 22.1 ± 6.8 units/ml./minute with a normal range of 5 to 40 units, while that of serum glutamic-pyruvic transaminase (SGP-T) has a mean value of 16.0 ± 9.0 units/ml./minute with a normal range of 5 to 35 units.

The large amount of enzyme in liver tissue, compared to the relatively insignificant concentration in normal serum, is associated with large increases of serum enzyme activity in response to minimal degrees of liver tissue injury. Acute liver cell injury as seen in acute infectious and homologous serum hepatitis results in impressive increments in the serum of both transaminases. Although the changes in the activity of the two enzymes parallel each other, the rise of SGP-T exceeds that of SGO-T. Peak levels of transaminases are usually found when the patient is the sickest. Extra-hepatic biliary obstructive jaundice is characterized by increments in transaminase activity from 80 to 250 SGO-T units and 100 to 350 SGP-T units; although both enzymes are altered in the same direction, the SGP-T activity usually exceeds the corresponding SGO-T activity. Cirrhosis when decompensated or active is frequently associated with an increase in SGO-T greater than the increment of SGP-T activity.

Serum transaminase levels are not elevated in patients with infectious, neoplastic, degenerative, reactive, allergic or congenital disease or pregnancy states, unless evidence of acute damage to the liver, heart or skeletal muscle is present. These latter clinical situations present little or no confusion diagnostically inasmuch as they appear in different clinical settings and are reflected by alterations in transaminase of appreciably different magnitude and serial change. In all of these clinical settings, whenever there is a rise in SGO-T there is consistently no elevation or an appreciably smaller elevation of SGP-T activity.

The measurement of SGO-T and SGP-T alterations has been found to be a useful tool in the diagnosis and study of acute hepatic disease and acute extrahepatic biliary obstructive jaundice. SGP-T appears to be more sensitive than SGO-T in reflecting acute hepatocellular injury. SGO-T alterations, however, more sensitively reflect active chronic hepatic disease. By the simultaneous measurement of SGO-T and SGP-T activity, it is possible in most cases to differentiate acute from active chronic liver cell injury. The quantitative and serial measurement of both enzymes permits accurate differential diagnoses of many clinical types of hepatic disease.

INFECTIOUS DISEASES

Application of the Fluorescent Antibody Technic to the Immunology of the Group A Streptococcus

By *George J. Friou*

The Coons technique for visual localization of antigen-antibody in tissues with fluorescein-conjugated antisera has been used to investigate localization of streptococcal antigens in mice. Antistreptococcus sera were prepared by immunizing rabbits with various preparations of group A streptococci. Soluble antigens were extracted from streptococcal cells disrupted by sonic oscillation. When injected intravenously there was localization in the glomerulus for a period of hours.

Certain antistreptococcus antisera localized in various tissues of normal mice. This localization is a specific property of these antistreptococcus rabbit sera. It suggests than an antibody is present for an antigen in mouse tissues. This type of localization of fluorescent antisera, and that due to specific localization of injected soluble antigen, were studied.

Host Resistance of Microbial Infection and Intoxication Unrelated to Specific Immunity

By *Edward W. Hook and Robert R. Wagner*. From the Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Various microorganisms and substances of microbial origin produce rapid and transient alterations in host susceptibility to infection. The action of these materials appears to be distinct from classical mechanisms of immunity. The present observations are preliminary results of studies designed to determine whether a general phenomenon underlies the action of these microbial substances in bolstering host defenses.

Mice injected intraperitoneally or intravenously with typhoid vaccine become highly resistant within 24 hours to intraperitoneal challenge with virulent typhoid bacilli. Protection can also be conferred against cerebral typhoid infection provided the preliminary inoculation of vaccine is made intracerebrally. Only minimal resistance to cerebral infection is induced by prior intraperitoneal or intravenous administration of vaccine.

A similar type of resistance to the neurotoxic action of influenza virus can be produced in mice. Convulsions and death which follow intracerebral inoculation of large doses of influenza virus are prevented by prior administration of subtoxic amounts of virus given by the same route. This resistance to influenza virus is rapid in onset and subsides within a few days; it is not dependent upon antibody formation. In addition, certain bacterial products, including typhoid vaccine, induce transient resistance to the neurotoxic action of influenza virus in mice. However, active infection of the brain by typhoid bacilli is not significantly altered by prior intracerebral inoculation of influenza virus.

KIDNEY

Re-examination of Water Diuresis, Including the Role of the Neurohypophysial Antidiuretic Hormone (ADH)

By *Abraham G. White*. Department of Medicine, Mount Sinai Hospital, New York City

Water diuresis following oral hydration presents several problems concerning the mechanisms (including the role of ADH) underlying (a) "lag" between maximal blood dilution and the attainment of peak diuresis; (b) magnitude of peak diuresis; and (c) the rate of decline of the diuresis curve following immediately after the peak. Current concepts of the role of ADH in water diuresis also do not explain these findings in diabetes insipidus: relatively low daily urine flow and excretion of hypotonic urine following osmotic loading.

One or 1.5 L. water were administered orally to human subjects, and 1.25% to 10% of body weight to rats by gavage. Studies of diuresis following oral or intravenous hydration of humans suffering from diabetes insipidus will be discussed.

Besides the usual measurement of the ensuing water diuresis (ml./min. vs. time), a plot was made of log $(a/a - x)$ vs. t where $a = 100\%$ of the administered water load, and $x = \%$ excreted at time, t. With this semi-logarithmic plot of water diuresis a straight line was obtained, beginning at approximately 60 min. and extending in many instances almost to the end of diuresis. In 2 patients with diabetes insipidus the plots were abnormal either in slope or shape. In the rat peak diuresis varied from 21.2 to 100.3 mcl./min./100 Gm. body weight as the administered load was increased from 1.25 to 10% of the body weight. Thus, the excretion of water becomes an exponential function at about the time described by previous investigators as that of maximal dilution of the blood. The peak of diuresis varies

with the water load administered, and does not represent a maximal rate of excretion if the urinary flow is measured in terms of the water load remaining in the body.

Physiologic Studies of Water Diuresis in the Nephrotic Syndrome

By *Mackenzie Walser and Jack Orloff*. National Heart Institute, Bethesda, Maryland.

Minimal urinary osmolality has been considered a criterion of renal diluting function, despite recognition that osmolality rises as solute excretion increases. The rate of excretion of solute-free water (C_{H_2O}) has been suggested as an alternative criterion. However, C_{H_2O} also varies with solute excretion. As reported previously, both in normal subjects and in patients with nephrogenic diabetes insipidus C_{H_2O} increases from 4–7% of filtration rate (GFR) during simple water diuresis to 10–18% of GFR during superimposed osmotic diuresis.

The relationship between C_{H_2O} and solute excretion was determined in 7 patients with the nephrotic syndrome, with varying degrees of renal insufficiency (GFR 13 to 153 ml./min.). The relationship noted in these patients resembled that observed in normals and in subjects with nephrogenic diabetes insipidus. The observed failure of the more severely ill patients to achieve a low urinary osmolality was related to a higher rate of solute excretion relative to the filtered load of solute. These results are consistent with the view that there may be a reduction in the number of functioning nephrons without alteration in their diluting ability.

In 3 patients the ratio of C_{H_2O} to GFR was higher than normal. Assuming that free water is formed by sodium reabsorption, this observation may be related to the increased sodium reabsorption in these patients. However, no correlation was observed between C_{H_2O} and the control rate of sodium excretion which varied from 1 to 100 mEq./min. Furthermore, acetazoleamide inhibition of sodium reabsorption was associated with higher ratios of C_{H_2O} to GFR than when diuresis was induced by mannitol. An alternative interpretation of relatively high C_{H_2O} is that glomerular dysfunction was preponderant in these patients.

The Graded Effect of Purified Vasopressin and Urinary Solute on the Excretion of Solute Free Water

By *Henry N. Wagner, Jr., Douglas G. Davidson and Jack Orloff*. National Heart Institute, Bethesda, Maryland.

Among the factors involved in the renal regulation of water excretion are the glomerular filtration rate (GFR), the rate of solute excretion and the activity of antidiuretic hormone (ADH). Studies were performed in normal dogs to delineate in a quantitative fashion the effect of the latter two variables on the excretion of solute-free water (C_{H_2O}). Variations in GFR were minimized by regulating the degree of bodily hydration. The relationship between urine flow and solute excretion was determined during physiological diabetes insipidus and during the infusion of differing amounts of purified arginine vasopressin, at various levels of solute excretion. The response to vasopressin was assessed by its effect on the excretion of solute-free water.

During endogenous ADH suppression an increase in solute excretion was associated with a rise in C_{H_2O}. The administration of increasing amounts of purified vasopressin (0.5, 1.0, 1.5, 2.0 and 5.0 mUnits/Kg./hr.) in separate experiments resulted in a progressively lower rate of solute-free water excretion at each dosage level, demonstrating a graded effect of the hormone. However, no significant difference was noted between the effects of 5 and 50 mUnits/Kg./hr. of vasopressin over the range of solute excretion examined. Urine osmolality in excess of that of plasma was effected by as little as 1.5 mU./Kg./hr. of vasopressin when solute excretion was 600 microOsm./minute but not when it was 2000 microOsm./minute. Five mU./Kg./hr. of vasopressin was sufficient to produce urine hypertonicity at all levels of solute excretion studied. The data are consistent with the view that the rate of excretion of solute-free water is determined by the integrated effect of excreted solute and ADH activity.

These studies afford an explanation for the observations that acute increases in solute excretion may be associated with a change in urine concentration from hypertonic to hypotonic.

The Renal Excretion of Intravenous 5% Sodium Chloride in Subjects with Renal Hypertension

By *W. Hollander and A. Chobanian*

The renal response to 300 cc. of intravenous 5% sodium chloride given at a rate of 10–12 cc./min. was studied in subjects with hypertension secondary to renal disease to determine whether patients with "renal hypertension" have the same disturbance in sodium excretion as that previously reported in patients with "essential hypertension."

Like the 15 subjects with essential hypertension, the 12 subjects with renal hypertension had significantly greater increases and higher rates of sodium excretion following the saline infusion than did the 10 normotensive individuals. In 2 hypertensive patients with unilateral kidney disease, sodium excretion became normal following nephrectomy and was associated with a return of the blood pressure, renal plasma flow (PAH clearance) and glomerular filtration rate (inulin clearance) to normal. Sodium excretion in the group was incon-

sistently related to the control sodium excretion, renal plasma flow and glomerular filtration rate. The differences in sodium excretion, as indicated by the ratio of sodium to inulin clearance, appeared to be due to a greater tubular rejection of sodium in the hypertensive than in the normotensive group.

We conclude, therefore, that subjects with "renal hypertension" have the same disturbance in sodium excretion following the infusion of 5% sodium chloride as those with "essential hypertension." The findings also suggest that common pathophysiologic factors are present in essential and renal hypertension.

Urinary Osmolar Concentration in the Hydropenic State as a Measure of Renal Tubular Function: A Preliminary Report.

By *Martin N. Frank, Leonard S. Dreifus, Fred Rarrick and Samuel Bellet.* Division of Cardiology and the Medical Wards of the Philadelphia General Hospital and the Robinette Foundation of the University of Pennsylvania School of Medicine, Philadelphia.

The ability of the kidneys to concentrate or dilute urine, as measured by specific gravity, has long been utilized as a method of testing renal tubular function. By present standards, the specific gravity is not an accurate or even direct measurement of tubular work, being affected significantly by temperature, pH, and various solutes such as urea, creatinine, sugar and protein.

A total of 80 patients, including 37 normals, 23 with essential hypertension, 13 with diabetes mellitus, and 7 with compensated rheumatic heart disease were selected for this study. In the latter 3 groups a diagnosis of renal disease was not substantiated by usual renal function studies. The blood urea nitrogen, Fishberg concentration test and total solute concentration (using the Fiske Osmometer for freezing point depression) were performed on all patients.

A fairly good linear correlation of urinary specific gravity and urinary osmolar concentration was present in the normal patients in the hydropenic state. On the other hand, patients with essential hypertension, diabetes mellitus and rheumatic heart disease exhibited a definite lack of correlation between urinary specific gravity and total solute concentration. The mean total solute concentration in the hydropenic state for the hypertensive, diabetes mellitus and rheumatic heart disease patients was 643 ± 180 mOsm./L., 647 ± 232 mOsm./L. and 610 ± 225 mOsm./L. respectively, as compared to 1076 ± 167 mOsm./L. for the normal group.

The essential hypertensive, diabetes mellitus and rheumatic heart disease patients exhibited a marked and early inability to concentrate urinary solutes. This tubular defect was often evident before routine clinical urinary function studies suggested renal impairment. Urinary osmolar concentration as measured by the total solute concentration may offer a relatively simple measurement of the early loss of renal tubular concentrating ability and serve as an index for the evaluation and prognosis in patients with renal impairment.

Renal Cortical Necrosis

By *David P. Lauler and George E. Schreiner.* Department of Medicine and Renal Laboratory, Georgetown University Medical Center, Washington, D. C.

Bilateral cortical necrosis has been considered a universally and rapidly fatal disease of unknown pathogenesis. Although the lesion occurs with greatest frequency in multiparae with abruptio placenta, cortical necrosis also has been reported in infants with diarrhea and adults with trauma, burns, periarteritis, Marfan's disease and a variety of infections and toxins. A similar lesion is produced experimentally by staphylococcal filtrate, E. coli endotoxin, killed streptococci, hog cholera virus vaccine, several vasoconstrictor substances and serotonin.

This study concerns 4 patients with cortical necrosis. Case 1 was an 18-year-old primipara with toxemia for pregnancy who was dialyzed once during 8 days of oliguria and died from acute hepatic necrosis and hyperkalemia. Case 2 was a 69-year-old male with automobile trauma who was dialyzed once during 24 days of severe oliguria and died with a ventricular arrhythmia. Case 3 was a 35-year-old multipara (11) with abruptio placentae who was dialyzed once during 10 days of oliguria and died with septicemia after 14 days of diuresis in excess of 1000 ml./day. Case 4 was a 32-year-old multipara with abruptio placentae and 3 dialyses during 32 days of oliguria.

Our experience with three cases of renal cortical necrosis surviving more than 24 days and the occurrence of a diuresis in case 3, suggest that with proper medical management, this disease need not be rapidly fatal and may even be compatible with survival as reported in one case by Gormgen, Iversen and Raaschou.

Kidney Function during Acute Tubular Necrosis: Clinical Studies and a Theory

By *William H. Meroney and Milton E. Rubini.* Department of Metabolism, Walter Reed Army Institute of Research, Washington, D. C.

The opinion is generally stated that the function of the kidney is passive during the oliguric phase of acute tubular necrosis. The major portion of the glomerular filtrate is considered to re-enter the extracellular fluid by back diffusion through discontinuous or inert tubular walls, and the small portion which escapes to the bladder is similar to an ultrafiltrate of plasma.

The present observations are inconsistent with this view and indicate that the oliguric kidney can

function actively in altering the composition of the glomerular filtrate in a direction favorable to the organism.

The osmolality, and the sodium and potassium concentrations in urine and plasma of 10 patients with oliguria following shock are presented. The osmotic U/P approximated unity. Invariably, urinary sodium was lower and potassium was higher than plasma concentrations. Independent of urinary volume, urinary sodium, initially high, decreased on successive days of oliguria. Urinary potassium varied inversely with sodium and increased progressively. Abrupt reversal in the downward trend of the urinary Na/K coincided with changes in plasma concentration effected by artificial hemodialysis, but the ratio did not return to former values. Thus sodium conservation and potassium excretion were partially independent of plasma concentration. Since the improvement in quality preceded the increase in quantity of urine in all patients, it suggested an adaptive response of those tubules already functioning rather than the contribution of urine by newly healed nephrons.

These results belie any suggestion that all tubules function as passive conduits during oliguria. It is proposed that the urine is largely a product of tubules which were spared the initial ischemic injury and are capable of rapid and progressive improvement in function when subjected to intense stimulation.

Renal Hemoglobin Transport: Glomerular Permeability and Tubular Reabsorption During Infusions of L-norepinephrine in Dogs.

By *Willoughby Lathem*. Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

The relationship between the transglomerular transport and renal tubular reabsorption of proteins is not clear. Conflicting evidence has suggested (a) that tubular reabsorption of hemoglobin may vary independently of glomerular permeability or (b) that an interdependent relationship may exist between these functions. In the present study an investigation of this relationship was undertaken during the administration of pressor doses of L-norepinephrine, which have been shown to accelerate urinary hemoglobin excretion, presumably by altering transglomerular transport.

Control and experimental observations were made in 10 anesthetized dogs. During the experimental studies an infusion of L-norepinephrine was administered intravenously at a rate sufficient to elevate mean arterial blood pressure 25–60 mm.Hg. One to two Gm. hemoglobin were given intravenously in a single rapid injection. From the relationship between hemoglobin excretion and the product of the glomerular filtration rate (creatinine clearance) and plasma hemoglobin concentration, the glomerular permeability to hemoglobin relative to creatinine (K) and the maximal rate of tubular reabsorption of hemoglobin ($T_{M_{Hgb}}$) were determined.

In the control studies K averaged .046 ± .011 and $T_{M_{Hgb}}$ averaged 2.20 ± 0.83 mg. per minute. During the administration of L-norepinephrine K and $T_{M_{Hgb}}$ increased in 8 animals, decreased in one and remained unchanged in one. The average change in K was .024 and in $T_{M_{Hgb}}$ 0.94 mg. per minute. These changes were significant.

These results suggest that an interdependent relationship between $T_{M_{Hgb}}$ and glomerular permeability may exist. The mechanism of these changes is not clear. L-norepinephrine may have accelerated transglomerular hemoglobin transport by reducing the velocity of glomerular blood flow, thus allowing more time for the transfer of hemoglobin molecules into glomerular filtrate.

RESEARCH METHODS

A Practical Ethylenediamine Tetra-Acetic Acid (EDTA) Method for the Determination of Calcium in Urine and Serum.

By *W. P. Myers and Rosalind Reydel*. Sloan-Kettering Institute, Memorial Center, and the Department of Medicine, Cornell University Medical College, New York City.

Disturbances in calcium metabolism have been observed in an increasing number of clinical situations, particularly in malignant disease both with and without detectable bone metastases. Urinary calcium measurements have been discovered to be a useful guide in the management of metastatic breast cancer and hypercalcemia has been noted in a wide variety of neoplasms.

In view of these clinical observations, a simplified technic for calcium analysis has become desirable. The most promising technics have involved the use of EDTA as a means of titrating calcium. The main difficulty has been the inadequate visual end-point when ammonium purpurate is the indicator and this has led to spectrophotometric determinations of the end-point.

In the method here reported calcium is titrated with EDTA in the presence of ammonium purpurate, using a visual end-point. The following modifications are employed which make the visual end-point readily ascertained: 1. Phosphate is removed by an anion exchange resin. 2. A double-tube titration technic is used so that one can titrate to the fully developed color in the second tube. 3. A daylight fluorescent screen, such as an x-ray viewing box, is used as a titrating background. Phosphate removal

from urine has been found by us and others to be essential for proper visualization of the ammonium purpurate end-point. A single analysis requires about 15 minutes.

The above modifications plus use of syringe pipettes previously described in other chemical analyses have resulted in a simple and workable method. Comparisons with the Fiske and Logan and Clarke and Collip methods were excellent and recoveries varied between 97–103%. It is hoped that this method will enable physicians to make greater use of quantitative calcium data in the care of patients.

The Use of Cineradiography with Image Intensification in Clinical Research

By *David B. Coursin, Herbert K. Cooper and F. Allan Hofmann.* Lancaster Cleft Palate Clinic, Lancaster, Pennsylvania.

The development of electronic means for amplifying the intensity of the light image produced by x-rays has opened a number of new horizons for study in clinical research. This advance now permits minimal amounts of radiation to be used for seconds at a time with resultant excellent visibility of parts under study. The Phillips Image Intensifier increases the light intensity of routine fluoroscopy level by one thousand times with resultant improvement of human eye visual perception from 2–3% to almost 100%. Selected positioning of the patient with respect to components of the apparatus further provides magnification of specific parts under study by a factor of 2.5 times.

Recordings of function and activity during observation can be made on 16 mm. film running as rapidly as 120 frames per second. Additional provisions have been made for simultaneous sound recordings that are synchronized with the picture sequence. This technic can be applied to problems of respiration, speech, cleft palate, angiocardiography, gastrointestinal and genitourinary motility, cardiac activity, and joint motion.

THERAPEUTICS

The Treatment of Acute Barbiturate Intoxication with B-B-methylethylglutarimide (N P 13-Megimide).

By *George E. Schreiner, Renato D. Kovach and Leonard B. Berman.* Department of Medicine, Renal Laboratory, Georgetown University Medical Center, Washington, D. C.

Glowing reports have appeared in the British literature since the introduction of B-B-methylethylglutarimide as a "specific barbiturate antagonist." Very little published data has been corroborated by blood barbiturate levels and there has been no published experience in this country.

This is a preliminary report on the use of Megimide in acute barbiturate intoxication.

One case ingested 80 Secobarbital capsules and was admitted to another hospital comatose, cyanotic, bradypneic (8/ min.) and hypotensive (80/50). Vigorous treatment for 10 hours with picrotoxin (> 250 mg.) and Metrazol failed to elevate the level of anesthesia. Blood level of secobarbital on transfer was 5.4 mg.%; administration of 1.0 grain Megimide I.V. over a 2-hour period restored thoracic breathing, corneal, knee and ankle reflexes and was followed by spontaneous movements and recovery. Another patient claimed to have ingested 3 to 5 Gm. Nembutal and 30 hours of vigorous therapy with caffeine, metrazol and ritalin failed to lighten the deep level of anesthesia. Blood barbiturate level on transfer was 2.0 mg.%. 0.2 grain Megimide restored corneal, ankle and patellar reflexes in 20 minutes and 1.25 Gm. in two hours was followed by spontaneous movement.

Preliminary experience indicates that Megimide is a promising drug for the following purposes: 1. Diagnosis of the depth of anesthesia by the dose required for biological effect. 2. Maintenance of a lighter state of anesthesia in moderate barbiturate intoxication. 3. Emergency therapy for bradypnea in severely poisoned patients being prepared for the definitive therapy of drug removal by dialysis.

PROGRAM, WESTERN SECTION

American Federation for Clinical Research

Wednesday-Thursday, January 30-31, 1957

Golden Bough Theater, Carmel, California

Dr. Sherman M. Mellinkoff, Chairman, Presiding

Presentations will be limited to ten minutes

WEDNESDAY, JANUARY 30, 1957

1:30 p.m.

1. **Erythropoietic Activity in the Plasma of Polycythemic Patients.**
   A. N. Contopoulos, J. H. Lawrence,† R. K. McCombs and M. E. Simpson,** Berkeley.
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2. **Lobarspirometry; Effect on Body Position.**
   C. J. Martin, A. C. Young and John J. Koler,** Seattle.
   page 47

3. **Uric Acid Metabolism: The Oxidation of Uric Acid in Normal Subjects and Patients with Gout, Polycythemia and Leukemia.**
   Myron Pollycove, B. M. Tolbert, J. H. Lawrence† and Denham Harman,** Berkeley.
   page 38

4. **Arterial pH and the Regulation of Renal Acid Excretion.**
   Daniel H. Simmons and N. S. Assali, Los Angeles.
   page 43

5. **The Binucleated Lymphocyte Response to Low-Level Radiation Exposure in Man.**
   R. L. Dobson and M. Chupp,** Berkeley. (Introduced by J. H. Lawrence).
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6. **Infections with Members of the Bacteroides Fusiformis Group.**
   *Sydney M. Finegold and William L. Hewitt,** Los Angeles.
   page 41

7. **Cardiopulmonary Studies in Anoxia and Polycythemia Associated with Obesity.**
   Jerome E. Cohn, I. T. Kurita, Ramon Lange* and Hans H. Hecht,†* Salt Lake City.
   page 48

INTERMISSION (ten minutes)

8. **The Blood Disappearance of Radioactive Rose Bengal.**
   *William H. Blahd and Robert A. Nordyke,** Los Angeles.
   page 40

9. **Quantitative Analysis of the Radioactive Rose Bengal Test of Liver Function.**
   *Jerold M. Lowenstein,** San Francisco. (Introduced by David A. Rytand.)
   page 39

10. **A Quantitative Comparison of the Response to Exogenous Thyrotropin in Eythyroid and Thyrotoxic Human Subjects.**
    *Monte A. Greer and Herbert F. Shull,** Portland.
    page 38

11. **Studies on the Synthesis of Heme from Protoporphyrin.**
    Herbert C. Schwartz, George E. Cartwright* and Maxwell M. Wintrobe,†* Salt Lake City.
    page 29

12. **The Use of I^{131}-Labeled Triolein in the Detection of Steatorrhea.**
    *Morton I. Grossman and Paul Jordan,** Los Angeles.
    page 40

THURSDAY, JANUARY 31, 1957

9:00 a.m.

(*Short Business Meeting Precedes Scientific Papers*)

1. **Evaluation of Mitral Valve Insufficiency in Dogs: Electric Analog Simulation of Radioisotope Dilution Data.**
   Daniel Hayden, Wayne Garrett,* P. Jordan* and Rex L. Huff*, Seattle.
   page 34

2. **Phonocardiographic Features of Mitral Stenosis with Moderate and Severe Mitral Insufficiency.**
   Thomas F. Leo and Herbert N. Hultgren, San Francisco.
   page 34

3. **Distribution of Fe^{59}-Tagged Erythrocytes in Centrifuged Specimens as a Function of Cell Age.**
   E. Raymond Borun, William G. Gigueroa and Seymour M. Perry*, Los Angeles.
   page 29

4. **Platelet Loss in Operations with an Artificial Heart-Lung Apparatus.**
   Herbert A. Perkins, John J. Osborn* and Frank Gerbode,** San Francisco. (Introduced by William P. Creger.)
   page 35

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*By invitation
†Senior Member

5. **Studies on the Effect of Sodium Phytate (Sodium Inositol Hexaphosphate) in States of Hypercalcuria.**
   *Felix O. Kolb and Nancy F. Leveton,** San Francisco.
   page 37

6. **The Identification of the C-Reactive Protein and Various Antibodies in the Serum Gamma Globulin Obtained by Continuous Flow Paper Electrophoresis.**
   *R. J. Roantree,** *F. A. Pezold** *and Lowell A. Rantz,†* San Francisco.
   page 42

INTERMISSION (ten minutes)

7. **The Renal Clearance of Phosphate; the Influence of Blood Glucose and Serum Phosphate Levels.**
   *Elston R. Huffman, Charles J. Hlad** *and H. Elrick,** Denver.
   page 44

8. **An Analysis of the Dependence of Serum Osmolarity on the Serum Sodium Concentration.**
   *L. W. Birkenfeld,** *M. P. O'Meara,** *J. Leibman** *and I. S. Edelman,* San Francisco.
   page 37

9. **The Inaccuracy of the Water-Filled Spirometer in the Measurement of the Maximum Breathing Capacity.**
   *William W. Stead, Herbert S. Wells,** *N. L. Gault** *and John Ognanovich,** Minneapolis.
   page 47

10. **The Methyl Green-Pyronin Differential Nucleic Acid Stain as an Aid in the Study of Blood and Marrow Smears.**
    Seymour Perry, Los Angeles.
    page 33

11. **Hepatic Blood Flow, Oxygen Consumption and Wedge Pressure in Cirrhosis Before and After End-to-Side Portacaval Anastomosis.**
    *Allan G. Redeker,** *Herman M. Geller** *and Telfer B. Reynolds,* Los Angeles.
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Advance Reports Submitted to the Annual Meeting of the Western Section of the American Federation for Clinical Research

Golden Bough Theater, Carmel, Calif. · Wednesday-Thursday, January 30–31, 1957

BLOOD

Studies on the Synthesis of Heme from Protoporphyrin

By *Herbert C. Schwartz, George E. Cartwright, and Maxwell M. Wintrobe.* Department of Medicine, University of Utah College of Medicine, Salt Lake City.

By the use of an in vitro cell free system it has been possible to demonstrate that the insertion of iron into protoporphyrin is enzyme dependent. This report is concerned with a description of several characteristics of this enzyme(s).

The incorporation of radioiron into heme was used as a measure of heme synthesis in an avian erythrocyte hemolysate. The hemolysate, 5×10^{-5}M protoporphyrin, carrier iron and radioiron were incubated at 37°C. for 4 hours. The radioactivity in the heme was determined and expressed as the percentage of radioactivity added.

In a typical experiment the hemolysate gave 13.5% uptake. After centrifugation of the hemolysate at 1000 g for 30 minutes the residue gave 10.9% uptake. Homogenization of the hemolysate, after it had been made isotonic with KCl, gave 45.3% uptake. After centrifugation of the homogenate at 100,000 g for 1 hour, the supernate gave 23.6% uptake. The hemolysate without protoporphyrin gave less than 2% uptake. Protoporphyrin without the hemolysate gave less than 1% uptake. Maximum synthesis was obtained when the system was buffered with 0.1 M Tris (pH 7.4). In the presence of 0.1 M phosphate buffer, synthesis was not maximal, probably because of the binding of iron by phosphate.

These studies indicate that the synthesis of heme from protoporphyrin is enzyme dependent and a simple system has been developed with which it is possible to study factors that influence this reaction.

Distribution of Fe^{59}-Tagged Erythrocytes in Centrifuged Specimens as a Function of Cell Age

By *E. Raymond Borun, William G. Figueroa and Seymour M. Perry.* V.A. Hospital and the Department of Medicine, University of California Medical Center, Los Angeles.

Previous investigators have shown that Fe^{59} is incorporated into immature erythrocytes and that these tagged cells have a relatively low density when they first appear in the circulation. The present study demonstrates an increase in relative density of older Fe^{59}-tagged erythrocytes and suggests two applications of this information.

Eight hematologically normal subjects received an intravenous tracer dose of Fe^{59}. Serial blood specimens from each subject were centrifuged in plastic tubes and placed in a liquid freezing mixture. The portion of the tube containing frozen erythrocytes was cut into 4 equal layers and Fe^{59} activity was determined in an aliquot from each layer.

Fe^{59} activity was highest in the top layer and lowest in the bottom layer of erythrocyte specimens drawn within 15 days after administration of the isotope, decreased in the top layer and increased in the bottom layer between 15–90 days, and decreased in the bottom layer after 90 days. The ratio $\frac{\text{top layer } Fe^{59}}{\text{bottom layer } Fe^{59}}$ was greater than one

during the first 30 days, less than one between 60–120 days, and then again became slightly greater than one.

The increasing proportion of Fe^{59} in the bottom layer indicates a progressive increase in relative density of tagged erythrocytes with age. This density gradient can be used to separate erythrocyte populations of varying mean age for the study of other physical and chemical changes associated with normal in vivo aging. The decrease in Fe^{59} concentration in the bottom layer after 90 days is consistent with the expected disappearance of high density senescent cells; the midpoint of this decrease provides an estimate of the mean life span of senescent tagged cells ("potential" life span). This estimate was 103, 119, and 135 days in three subjects.

The In Vivo Survival of Cr^{51}-Labeled Erythrocytes Following Prior In Vitro Exposure to 5% Dextrose and 0.225% NaCl

By *Joseph R. Bove, Richard S. Stemler and Franklin G. Ebaugh, Jr.* Department of Pathology, Dartmouth Medical School, Hitchcock Foundation and Hitchcock Clinic.

The effect of prior in vitro exposure to 5 Gm. % dextrose and 0.225 Gm. % NaCl (solution I) on in vivo survival of human erythrocytes has been studied because of a series of hemolytic transfusion reactions with indirect Coombs test compatible blood exposed to this solution before transfusion. Red cells exposed to this solution for 3 hours increased to 146 ± 8% of their original volume; this swelling was accompanied by an increase in the erythrocyte glucose from the normal value of 5 millimols to 85–135 millimols per liter. A negligible loss of potassium (3%) occurred from the red cells during this period. The Cr^{51} in vivo survival 10 minutes after injection of a mixture of one part ACD blood to 19 parts of solution I was 90–120% after prior in vitro exposure to this solution for ½ hour; 50% after 1 hour; 20% after 1½ hours; 15% after 2 hours; and 8% after 3 hours' exposure. The Cr^{51} in vivo survival of one part ACD blood exposed to 2 parts of solution I for 3 hours prior to transfusion revealed an inconsistent pattern of from 21–115% in vivo survival, with no correlation between in vivo survival and the increased RBC volume. However, in vivo survival was inversely correlated with the increase in RBC volume of blood first exposed to solution I for 3 hours and then equilibrated with normal plasma 4 to 10 minutes. The critical cell size was 170 ± 10% of that in ACD blood. The decrease of in vivo survival caused by exposure to solution I for 3 hours was not corrected by a subsequent 1-hour exposure of the RBC to plasma before transfusion.

Erythropoietic Activity in the Plasma of Polycythemic Patients

By *A. N. Contopoulos, J. H. Lawrence, R. K. McCombs and M. E. Simpson.* Donner Laboratory and Donner Pavilion, and Institute of Experimental Biology, University of California, Berkeley.

Studies in this laboratory have shown an increased rate of red cell production in polycythemia vera. Whether this increased rate is due to the presence of a humoral erythropoietic factor, which has more recently been demonstrated, is not known.

The existence of circulating erythropoietic stimulants in the plasma of normal subjects and patients with polycythemia vera, stress polycythemia, and other blood dyscrasias was tested by the effect of injections of extracted plasma on the erythropoiesis of hypophysectomized recipient rats. Heparinized blood was collected from individual human beings, centrifuged, and the plasma aspirated into vials. The pH of the plasma was adjusted to 5.5 with 1N HCl, and the plasma was boiled for five minutes in a water bath. The precipitate was discarded by centrifugation, and the supernatant adjusted to pH 7.0 with 0.1 N NaOH, dialyzed against distilled water at 0° C., and afterward lyophilized. The dry substance from the individual samples was dissolved in saline in a concentration of 30 mg./cc. and injected into hypophysectomized rats 45 days after operation in doses of 1 cc./day/rat for 14 days. At the end of the injection period, red cell volumes were determined (Fe^{59} method). The plasma from five polycythemia vera patients showed significant erythropoietic activity as judged by a 25% increase in total circulating red cell volume and hemoglobin.

These results were confirmed by the use of the iron uptake of circulating erythroid elements as described by Hennessy and Huff and by Fried, et al. The iron uptake of blood of hypophysectomized rats, when injected 10 days postoperatively with 30 mg./day/rat for 2 days, was increased to significantly higher levels than those of noninjected controls, or of hypophysectomized rats injected with plasma from normal human beings, or of patients with blood disturbances (including stress polycythemia) other than polycythemia vera.

Determination of Urinary Excretion of Radiocobalt-Labeled Vitamin B_{12} by Cobalt Sulfide Precipitation

By *David S. Kinnory, Ervin Kaplan, Y. T. Oester and Albert A. Imperato.* Radioisotope Service, V.A. Hospital, Hines, Illinois.

Radiocobalt-labeled vitamin B_{12} has consider-

ably simplified the diagnosis of vitamin B_{12} malabsorption syndromes. In the Schilling test it is useful for diagnosing and differentiating pernicious anemia from other macrocytic anemias of liver disease and sprue. A need exists, however, for improving the counting efficiency and ability to discriminate between various radioisotopes in measuring low-level radioactivity of large volumes of urine. This prompted us to develop the more specific and sensitive method of cobalt sulfide precipitation for the determination of urinary radioactive cobalt.

The cobalt sulfide precipitation procedure is performed in the hood. It utilizes 1000 ml. of the patient's 24-hour urine, to which are added 4 ml. of 1 M cobalt chloride as carrier, 300 Gm. of NH_4Cl to prevent colloid formation of cobalt sulfide, and 5 ml. of n-octyl alcohol to reduce foaming. The mixture is then heated to boiling, 6 ml. of Merck reagent $(NH_4)_2S$ are added and, after cooling, the cobalt sulfide precipitate is isolated by suction filtration. The precipitate with filter paper is packed in the bottom of a Lusteroid tube and counted in the well-type scintillation counter against the dose standard. The radioactivity recovered in the cobalt sulfide from two successive precipitations compares with that obtained by urine evaporation. In the second precipitation, the addition of NH_4Cl and n-octyl alcohol is omitted.

This procedure has a two-fold advantage. (1) Its high counting efficiency permits the use of only a 0.05 microcurie dose of radiocobalt-labeled vitamin B_{12} with a high counting rate of 2700 c.p.m. for a 10% recovery using a 2″ x 2″ sodium iodide crystal. (2) Its relative specificity makes it possible to administer the Schilling test to patients who have previously been given other radioisotopes and are still excreting radioactivity not precipitable from urine by sulfide ions.

Studies on the Agglutinin and Hemolysin of Leukemic Leukocytes

By *Bernard Pirofsky*. Division of Experimental Medicine, University of Oregon Medical School, Portland, Oregon.

Leukocytes from 13 cases of various types of leukemia were isolated, homogenized, and serially extracted in 0.88 M sucrose, 0.14 M NaCl, and 2.0 M NaCl with separation of extracts by ultracentrifugation. Thirteen out of 13 0.88 M sucrose extracts, 7 of 13 0.14 M NaCl extracts, and 12 of 13 2.0 M NaCl extracts were found to contain an agglutinin and hemolysin active against trypsinized and normal O Rh negative erythrocytes. The hemolysin and agglutinin was active at 37°C. with a variable titer at 6°C., was spontaneously inactivated in 2 months even with freezing, and would induce choleglobin formation. Detailed studies of this hemolysin and agglutinin revealed the following:

(1) The hemolysin and agglutinin is dissimilar from coating antibody eluted from Coombs positive erythrocytes obtained from patients with acquired hemolytic anemia complicating leukemia.

(2) The hemolysin and agglutinin does not appear to be β-glucuronidase.

(3) The hemolysin and agglutinin does not function as an anti-H agglutinin.

(4) Widely separated areas of hemolytic and agglutinating activity were found by elution of extracts after electrophoretic separation.

(5) There are many similarities between this agglutinin and hemolysin and lysin-inhibitor complexes isolated from normal and tumor tissues. However, the hemolysin and agglutinin of leukemic leukocytes is found in microsome-free extracts and is destroyed by further extraction with organic solvents.

(6) There is a normal serum inhibitor of this hemolysin and agglutinin. The inhibitor is thermostable at 56°C. for 30 minutes, is not dialyzable, is not absent from leukemic sera, and does not permanently inactivate the hemolysin and agglutinin.

Clinical Evaluation of Chlorambucil

By *Edward Shanbrom, Alfred G. Knudson, Jr. and Stanley Rappaport*. Lymphome-Leukemia Section and Pediatrics Section, Hospital for Tumors and Allied Diseases, City of Hope Medical Center, Duarte, California.

Fifty patients with lymphomas, leukemias, and multiple myeloma have been treated with Chlorambucil (CB 1348).

Distinct improvement, both clinical and hematologic, occurred in 40 adults with chronic lymphocytic leukemia, acute monocytic leukemia, and lymphomas.

Subjective improvement (increase in appetite, sense of well-being, decrease in bone pain and gain in weight) occurred in 4 of 6 patients with multiple myeloma who no longer responded to other forms of therapy.

Four children with lymphomas have improved on doses of Chlorambucil that were considerably larger per kilogram than the adult doses used. In adults, the dose bears a direct relationship to tumor size.

The drug is usually well tolerated. Gastrointestinal symptoms occurred in 15 patients. Three patients had pruritis and skin eruptions. Mental depression occurred in 3 patients. Bone marrow depression of varying degrees occurred in 20 patients, but was reversible in all. Symptoms associated with this depression were mild.

Comparison of Therapeutic Effects of 6-Methyl Mercaptopurine, 6-Chloropurine, 6-Thioguanine and 6-Mercaptopurine in Human Acute Leukemia

By *D. R. Hales, R. W. Jerner, B. E. Hall, F. M. Willett, J. Franco and T. V. Feichtmeir.* Department of Medicine, Stanford University School of Medicine, and San Francisco V.A. Hospital. (Aided by a grant from the National Institutes of Health.)

The anti-purine compounds have been shown to be more effective than other anti-leukemic agents in inducing remission in acute leukemia in adult human beings. While 6-mercaptopurine has been shown to be far more effective in adult patients than anti-folic or steroid compounds, agents that might prove to be superior to 6-mercaptopurine have also been investigated. Other agents in the anti-purine series studied to date include 6-thioguanine, 6-methyl mercaptopurine, and 6-chloropurine.

The results of treating 26 adult patients having acute leukemia with 6-mercaptopurine, 8 patients with 6-thioguanine, 16 patients with 6-methyl mercaptopurine and 7 patients with 6-chloropurine will be compared. On the basis of available evidence, it appears that 6-thioguanine is no more effective as an anti-leukemia agent than 6-mercaptopurine, and is slightly more toxic; 6-methyl mercaptopurine is less effective than 6-mercaptopurine; and 6-chloropurine is as good as, and perhaps better than, 6-mercaptopurine.

The Relationship Between Leukocytes and Fever in Leukemia

By *A. I. Braude, M. Zalesky and J. M. Beck.* Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas.

Injection of bacterial pyrogen is followed successively by the following changes: (1) its localization in buffy coat; (2) neutropenia; and (3) fever. These changes, and the discovery by Bennett and Beeson of endogenous pyrogen in granulocytes, suggest the hypothesis that fever results from interaction between bacterial pyrogens and granulocytes. Because this hypothesis implies that deficiencies of granulocytes should diminish the febrile response, the action of pyrogen was investigated in leukemics. Each patient received intravenously 50,000,000 dead typhoid-paratyphoid bacilli, and their effect was noted on temperature and circulating leukocytes. Twelve non-leukemic controls received identical injections. Indices for quantitative comparison of fevers were computed by measuring the area under 10-hour temperature curves.

All nonleukemics sustained high fevers, with an average peak of 102.4°F. at 5 hours and a mean fever index of 38.4. Four patients with chronic granulocytic leukemias also experienced high fevers with an average peak of 103.7°F. at 3 hours and a mean index of 37.4. Among 6 with lymphocytic leukemia, however, temperatures in 4 did not exceed 99.6°F. and the fever index for 6 patients was only 9.3. Two patients with lymphocytic leukemia experienced brief elevations above 102°F.; one had pyelonephritis and another received 5 transfusions of blood, a source of granulocytic pyrogen.

In normals and patients with granulocytic leukemias, fever was characteristically preceded by a fall in granulocytes (neutrophils and basophils) to 50–80% of the original count within 90 minutes. Original granulocyte levels were soon regained in leukemics and exceeded in normals. In 5 patients with chronic lymphocytic leukemia (total lymphocytes 70,000–450,000), granulocytes were too sparse for accurate counts, but leukemic lymphocytes fell irregularly.

These results indicate that deficiencies of granulocytes reduce the febrile response to pyrogens and support the concept that granulocytes, whether normal or leukemic, may be required for production of fever.

The Methyl Green-Pyronin Differential Nucleic Acid Stain as an Aid in the Study of Blood and Marrow Smears

By *Seymour Perry.* Department of Medicine, School of Medicine, University of California, and the V.A. Hospital, Los Angeles.

Desoxyribosenucleic acid is confined to the nucleus in mammalian cells, including those of the bone marrow, while ribosenucleic acid is found predominantly in the cytoplasm and nucleoli. In view of recent work which has established the specificity of methyl green for desoxyribosenucleic acid and of pyronin Y for ribosenucleic acid, we have adopted a modification of these stains to study peripheral blood and bone marrow smears of normal individuals and patients with various diseases.

The staining intensity of the mature lymphocyte does not vary and therefore serves as a control against which changes in RNA of other cells are graded. With the exception of plasma cells and lymphocytes, intensity of RNA staining decreases as cells mature. DNA remains constant regardless of cell age but varies with the morphologic type. Mature neutrophils, monocytes, lymphocytes, and plasma cells and their precursors, as well as the erythrocyte precursors, are more easily distinguished by this technic than by more conventional methods. The segmented neutrophils in the periph-

eral blood of normal individuals vary in age, although they appear morphologically identical by the Wright's stain.

Four hundred eleven patients with 67 diseases were studied. Highest RNA values were obtained in acute and chronic leukemias, bacterial infections, and pernicious anemia in relapse. Beneficial therapy in leukemia failed to decrease the intense RNA staining. The marked pyroninophilia of plasma cells in multiple myeloma was unaffected by treatment with steroids. A therapeutic response in pernicious anemia resulted in a return of the RNA staining to normal. Bacterial infections demonstrated increased RNA in the peripheral blood leukocytes regardless of the height of the count or the degree of immaturity. The intensity of RNA staining became normal with recovery.

With the use of this stain, it is possible to study changes in the nucleoprotein of blood cells as a consequence of the basic disease process. In addition, differentiation of the various blood cells, especially the precursors, is more readily accomplished than with the usual stains.

Interaction Between Desoxyribonucleic Acid and Quinacrine: Effect on DNase Susceptibility

By *N. B. Kurnick and Irma E. Radcliffe.* V.A. Hospital, Long Beach, and the Department of Medicine, University of California, Los Angeles. (Aided by grants from the American Cancer Society, National Institutes of Health, and the Los Angeles County Heart Association.)

Whereas the therapeutic value of quinacrine (atabrine) in systemic lupus erythematosus is doubtful, it has been shown to interfere with the L.E. cell phenomenon in vitro. Since the L.E. cell phenomenon involves desoxyribonucleic acid (DNA) and desoxyribonuclease (DNase), we have investigated the effect of quinacrine on this system.

We have found, by viscosimetric methods, that quinacrine forms a compound with polymerized DNA that is not susceptible to DNase depolymerization. The suppression of depolymerization is not due to inhibition of the enzyme, since the inhibition is independent of the enzyme-quinacrine concentration. It is dependent on the quinacrine-DNA ratio. The minimum quinacrine-DNA ratio at which inhibition is complete corresponds to the stoichiometry of the quinacrine-DNA compound as determined by dialysis, viscosimetry, and ultra-centrifugation.

Quinacrine displaces methyl green from the methyl green-DNA compound and also prevents access of DNase to DNA. This suggests common sites of attachment.

Quinacrine apparently prevents the L.E. cell phenomenon, then, by reacting with the nuclear DNA so as to prevent depolymerization by DNase. This is quite different from the action of the specific intracellular protein inhibitor of DNase, which reacts with the enzyme and not the substrate. It may be that the former mechanism is deleterious to the cell and is related to the mechanism of its antiplasmodial action.

CARDIOVASCULAR SYSTEM

The Reliability of the Determination of Cardiac Output in Man Using the Fick Principle

By *Arthur Selzer and Robert Sudrann.* Medical Service, V. A. Hospital, and the Department of Medicine, Stanford University School of Medicine, San Francisco. (Aided by a grant from the San Mateo Heart Association.)

The "direct Fick method" of determining cardiac output during cardiac catheterization has been subjected to some criticism, especially in anoxemic states, in spite of being generally considered the standard method and a yardstick against which other methods are checked. It is noteworthy that the reproducibility of its results has only been tested in two small series of cases, including only a few patients with cardiac disease.

In this study, two separate determinations of cardiac output within a half-hour period were performed in a series of 120 patients. The cases were divided into four groups: A. Group of 30 individuals with normal output; B. Group of 41 patients with cardiac disease and a moderate reduction in cardiac output; C. Group of 22 cardiac patients with a severe reduction in cardiac output; and D. Group of 27 patients with hypoxemia. The reproducibility of the results was tested separately in each group for determination of oxygen consumption, the arteriovenous oxygen difference and the cardiac output.

The results showed a significant difference between the reproducibility of oxygen consumption and of arteriovenous difference: in 75% of the entire series, oxygen consumption fell within a 10% variation range, and in 43%, within a 5% variation range. Arteriovenous oxygen difference fell in 86% of cases within the 10% range and, in 66%, within the 5% range. No significant difference was found among the four groups.

It is concluded that the reliability of the Fick method of determining cardiac output is satisfactory both in normal and abnormal cases. In serial studies of changes in cardiac output, the arteriovenous difference appears to be a more reliable index than the cardiac output as a whole. With the use of duplicate determinations, changes in arteriovenous difference are capable of detecting even small variations in cardiac output.

Phonocardiographic Features of Mitral Stenosis with Moderate and Severe Mitral Insufficiency

By *Thomas F. Leo and Herbert N. Hultgren.* Department of Medicine, Stanford University School of Medicine, San Francisco.

Most candidates for mitral valvotomy today are selected by clinical study alone. Therefore, the value of phonocardiography in predicting the degree of mitral regurgitation has been assessed. Phonocardiograms of two groups of patients were compared. One group had mitral stenosis with moderate regurgitation; therefore, valve fracture was not performed or was ineffectual. The unexplored group had obvious predominant severe mitral insufficiency determined by clinical and laboratory studies. A Sanborn Twinbeam phonocardiograph was employed, and simultaneous reference tracings of precordial impulses, carotid and jugular pulses, and electrocardiograms were recorded.

In patients with surgically significant, moderate moderate mitral insufficiency the following was observed: The first sound at the apex was delayed, as in pure stenosis; with one exception it was of normal or reduced amplitude. Moderately loud apical systolic murmurs were present in all patients. Inaudible opening snaps and audible protodiastolic gallops were recorded in 3 of 6 patients. Protodiastolic gallops preceded by rapid outward precordial movements were common and constituted a valuable sign of significant mitral insufficiency. These gallops were often misinterpreted clinically because of their variation in intensity, confusion with second sounds or opening snaps, or inclusion in the beginning of diastolic murmurs. Protodiastolic murmurs were present in all cases. Their delayed onset, frequent initiation by a gallop and short duration were distinctive features.

Patients with severe mitral insufficiency had loud gallops but no opening snaps. A few had no diastolic murmurs.

In patients with irregular rhythm, the intensity of the systolic mitral murmur did not vary in proportion to the duration of the preceding diastole. This is a useful clinical sign, differentiating this murmur from that of aortic stenosis, which may occasionally be transmitted to the apex.

Phonocardiography affords an accurate method of assessing the degree of mitral insufficiency in rheumatic heart disease and therefore may obviate more elaborate technics.

Evaluation of Mitral Valve Insufficiency in Dogs; Electrical Analog Simulation of Radioisotope Dilution Data

By *Daniel Hayden, Wayne Garrett, P. Jordan and Rex L. Huff.* Radioisotope and Surgical Services, V.A. Hospital, and the Departments of Medicine and Surgery, University of Washington, Seattle.

This investigation of mitral valve insufficiency was undertaken because of the possibility that radioisotope dilution technics would afford a means for quantitating reverse blood flow from left ventricle to left atrium both in the experimental animal and in man.

Activity-time curves have been obtained by

injecting I^{131} human serum albumin into the jugular vein of dogs and sampling simultaneously from both pulmonary artery and left atrium. Analysis of the data obtained has been achieved by proposing a circulatory model and setting up the electrical analog of this model on an analog computer. Data from the pulmonary artery are fed into the computer and the values of mixing pool turnover rates adjusted until the experimental data curve for the left atrium has been duplicated by the computer. In this way, functional mixing volumes for the lung, left atrium, and left ventricle have been calculated as well as the turnover rates between these pools.

Fifteen experiments have been made on open-chested dogs, 8 of which were normal. The "normals" showed a reverse flow of approximately 2–3 ml./sec. Seven dogs had varying degrees of mitral insufficiency induced by severing the chordae teninae of the mitral valve. Analysis of the data from the latter group showed reverse flow rates from left ventricle to left atrium of 10–40 ml./sec.

Future work includes obtaining additional information regarding the blood mixing pools and turnover rates of the cardiopulmonary circulatory system, as well as developing the technics described for the estimation of mitral insufficiency to the point where they can be applied clinically.

Aortic Grafts Stored in Alcohol

By *W. G. Dixon*. Provo, Utah. (Aided by a grant from the Utah Heart Association.)

The purpose of this investigation has been to determine the value of alcohol as a vessel preservative for homografts and heterografts.

The method of study has been to graft alcohol-stored aortas taken from dogs, pigs and humans and substitute them for the abdominal and thoracic aorta of dogs. The chest was usually chosen as the site of implantation, even though it was more technically difficult than the abdomen, because Kanar has shown that there is more calcification and atheromatous degeneration in thoracic than abdominal grafts. The alcohol was 70% isopropyl in most cases, ethyl being used in only a few because of tax problems. Anti-coagulants were not used. Photographs and measurements were taken at the time of implantation and sacrifice. Aortograms were done about every 6 months. If the aortograms showed abnormalities, the dogs were sacrificed at that time; otherwise, survivors were kept for at least a year. Microscopic sections were made of the host-graft junction and prepared with H. & E. and Weigert stains.

In 18 homografts there were 9 operative deaths and 9 survivors. Of these 9 survivors there were 4 good results, 4 bad results, and one pending.

In 8 heterografts there were 3 operative deaths and 5 survivors. Of these 5 survivors there was one good result and 4 bad results. Grafts were stored in alcohol an average of 82 days prior to implantation. All survivors were observed an average of 180 days. The good results were observed an average of 320 days.

Alcohol-stored grafts, especially the heterografts, have not been as consistently successful as those reported by others, but because of the simplicity of storage and the frequent good results, further investigation seems worth while.

Platelet Loss in Operations with an Artificial Heart-Lung Apparatus

By *Herbert A. Perkins, John J. Osborn and Frank Gerbode*. Department of Medicine, Stanford University, San Francisco.

Marked thrombocytopenia occurs during operations with an artificial heart-lung machine and may limit the time during which the procedure can be safely used. The factors involved were investigated in mongrel dogs. Platelet counts were done by a direct method, which resulted in an average count of 332,000 in 29 normal dogs.

In 28 operations the platelet count dropped to 62,000 or lower in four instances and was markedly depressed in all cases. Platelet counts were back to normal in anywhere from one hour to as long as six days.

The platelet count dropped only 10% during two hours of surgical stress prior to connection to the extracorporeal circuit. A further diminution of 48% occurred during the subsequent perfusion through the heart-lung machine for 30 minutes. No difference was apparent, whether vacuum-pressure or finger pumps were used, nor between bubble and film oxygenators. Platelets did not appear to be trapped in foam.

Loss of platelets by deposition on the foreign surface occurred to a slight extent, but neither this nor platelet lysis appeared to be an important factor inasmuch as recirculation through the pump-oxygenators alone for 30 minutes caused no appreciable decrease in the platelet count. The drop of 48% in the platelet count occurred only when the dog was in the circuit.

The major loss of platelets during operations with an artificial heart-lung machine thus occcurs during the period of perfusion, but is not due to loss of platelets in the machine. It may be that platelets are damaged by exposure to the machine and are then removed from the circulation by the dog. The situation is thus closely analogous to the thrombocytopenia that occurs with exchange transfusions using stored blood.

The Effect of Betaine on Human Serum Lipoproteins

By *Edward H. Strisower, Paul Elmlinger, Beverly Strisower and John Gofman.* Donner Laboratory, Radiation Laboratory, University of California and Napa State Hospital, Department of Mental Hygiene, Napa, California. (Aided by a grant from the International Minerals and Chemicals Corporation.)

The effect of betaine on serum lipoprotein and total serum cholesterol levels was studied in a group of 13 male and 3 female schizophrenic patients. The male patients received 15 Gm. betaine monohydrate daily for 4 weeks and the female patients received the same dose daily for 16 weeks. Blood samples were obtained on all patients before, during, and after the period of betaine administration.

It was found that betaine produced a marked elevation in the levels of the S_f°0–12 serum lipoproteins, ranging from 9–181 mg. per 100 ml. of serum (mean 53 mg.). No definitely significant changes were observed in the levels of the S_f°12–20, S_f°20–100, and S_f°100–400 serum lipoproteins, except for relatively large increases noted in the S_f°20–400 levels in two male patients who had abnormally elevated S_f°20–400 lipoprotein levels even before receiving betaine.

All patients showed an increase in total serum cholesterol levels, ranging from 2–56 mg. per 100 ml. of serum in the male patients (mean increase: 25 mg.), and from 81–139 mg. in the female patients (mean increase 106 mg.). No significant weight changes occurred during the experiment.

Our findings do not support those of Morrison, who reported a lowering of serum cholesterol values in patients given betaine. A more extensive study of the effect of betaine on serum lipids is now in progress and detailed findings will be reported at a later date.

Agents, like betaine, which provoke definitive alteration in serum lipoprotein levels deserve intensive study in the effort to understand over-all control mechanism of lipoprotein levels.

A Comparison of the Effect of β-Sitosterol, Gallogen and Safflower Oil on Serum Lipoproteins of Humans

By *John W. Farquhar and Maurice Sokolow.* Department of Medicine, University of California Medical Center, San Francisco.

This study was undertaken to determine the effect of a combination of β-sitosterol and Gallogen (diethanolamine salt of the mono-(+)-camphoric acid ester of alpha, 4-dimethylbenzyl alcohol) on the serum lipoproteins of humans and to compare its effect with that of a highly unsaturated vegetable oil.

Gallogen (a choleretic) and β-sitosterol have been reported to be synergistic in preventing hypercholesterolemia of cholesterol-fed pullets; their action in man has not been reported. The vegetable oil used (safflower oil) was chosen on the basis of its high content of linoleic acid (approx. 70%) and virtual absence of other constituents that might be expected to participate in the known serum cholesterol lowering effect of certain vegetable oils.

All experimental subjects had suffered from complications of atherosclerosis. Average age was 42. Placebo (control) periods preceeded and followed each experimental period in all patients. Eleven subjects have completed the first phase of the study; five have completed the second phase.

In phase I, following a 7-week placebo (control) period, subjects ingested 225 mg. of Gallogen and 6 Gm. of sitosterol t.i.d. for 10 weeks.

Phase II was consecutive and consisted of a 6-week placebo (control) period followed by an 8-week period during which each patient consumed 27 Gm. safflower oil t.i.d.

Constant dietary composition and body weight were the goal throughout these studies. In phase II, safflower oil was substituted in large part for dietary fat. The serum from weekly fasting blood specimens was analyzed for total cholesterol and for lipoprotein cholesterol (α = ALPC; β = BLPC) after separation by paper electrophoresis.

A prompt sustained decrease in mean total cholesterol and BLPC of 53 mg./100 ml. and 49 mg./100 ml. (16% and 25%) occurred during the sitosterol-Gallogen period. ALPC remained unchanged. The response to β-sitosterol and Gallogen was no greater than that reported previously in a similar study in which β-sitosterol alone was used. Phase II, at present incomplete, indicates that safflower oil causes a similar but more variable decrease in cholesterol levels.

ENDOCRINES AND METABOLISM

An Analysis of the Dependence of Serum Osmolarity on the Serum Sodium Concentration

By *L. W. Birkenfeld, M. P. O'Meara, J. Leibman and I. S. Edelman.* University of California Medical Center, San Francisco.

Previous reports of a lack of linear dependence of serum osmolarity on serum sodium concentration have been unsupported by rigorous statistical evaluation.

Measurements of serum osmolarity by freezing point depression and simultaneous serum sodium concentration determinations have been performed in 53 patients suffering from a variety of serious diseases, including acid-base disturbances. Serum osmolarity was corrected for measured glucose and NPN content and the sodium concentration was determined in serum water. The range of the uncorrected serum osmolarity values was 227–320 milliosmols/L. and the corresponding serum concentrations varied from 104–146 mEq./L. Statistical evaluation of the relationship of corrected serum osmolarity (¶) and the concentration of sodium in serum water $[NA]_s$ gave a highly significant correlation coefficient ($r = 0.96$ and $t = 173$). These data were tested for linearity by group rank analysis by the null hypothesis. The Fisher variance ratio, "F" was 1.31 while $F.05 = 2.23$ indicating no deviation from linearity. Calculation of an equation by least squares for the relationship of ¶ to $[NA]_s$ gave $¶ = 1.62[NA]_s + 28.4$. The standard deviation of ¶ for a given $[NA]_s$ was ± 5.2 milliosmols/L.

The osmolarity values of 51 patients fell within two standard deviations. The remaining two osmolarity measurements fell within 2.5 and 3.0 standard deviations, respectively. Neither of these 2 patients died within two weeks of the study. The variance of ¶ for a given value of $[NA]_s$, therefore, follows a normal distribution curve in spite of the fact that 10 patients died within two weeks of study.

These data indicate a linear dependence of serum osmolarity on the concentration of sodium in serum water when appropriate corrections are made for serum NPN and serum glucose. There was no evidence for the presence of osmotically active unidentified substances in the serum of patients ante-mortem.

Intermediary Metabolism in Diabetes

By *B. M. Tolbert, Martha R. Kirk and M. Pollycove.* Radiation Laboratory, Donner Laboratory and Donner Pavilion, University of California Berkeley. (Aided by a grant from the U. S. Atomic Energy Commission.)

The rate and cumulative excretion of labeled CO_2 following the injection of a single tracer dose of a carbon-14 labeled substrate has been used by the authors to study intermediary carbon metabolism in vivo. In this current research such respiration patterns have been measured to show the nature of the primary diabetic defect. In the experimental laboratory it has been shown that in severely alloxan diabetic rats, less than 10% of tracer quantity of glucose is oxidized to CO_2 in 2 hours, whereas in normal rats 25–35% is so oxidized. The rate of oxidation of fructose, lactate, and acetate in such animals is nearly normal. The glucose metabolism defect can be quickly and easily corrected by insulin injections. The oral sulfonamide drugs, tolbutamide and carbutamide, can also correct this metabolic defect in some cases, presumably by stimulating insulin production. The work shows that in advanced diabetes, the beta cells of the pancreas are apparently completely inactive with regard to insulin production and cannot be reactivated by the sulfonamide drugs. There is some evidence that the alpha cells of the pancreas may also be affected by these drugs.

In clinical studies the glucose-to-$C^{14}O_2$ respiration patterns of a variety of cancer and/or diabetic patients are being measured, some with and some without insulin or an oral sulfonamide.

Studies on the Effect of Sodium Phytate (Sodium Inositol Hexaphosphate) in States of Hypercalcuria

By *Felix O. Kolb and Nancy F. Leveton.* Metabolic Unit and the Department of Medicine, University of California School of Medicine, San Francisco.

Few measures short of dietary restriction were effective in reducing urinary output of calcium in states of hypercalcuria until the recent introduction by Henneman et al. of sodium phytate, which blocks its intestinal absorption. Since calcium phosphate stones are a frequent sequel to hypercalcuria, reduction of urinary phosphate by aluminum hydroxide gels, attempts to reduce colloids etc., have been in use. In order to reduce hypercalcuria we have used sodium phytate (E. R. Squibb and Sons) as a 15% aqueous solution in a simple syrup base in doses of 15 cc. three to four times daily in divided doses in a group of seven patients with established idiopathic hypercalcuria with recurrent renal lithiasis. Hyperparathyroidism was carefully excluded in all cases. The diet eliminated milk and cheese, but was otherwise nonselective. Duration of observation on phytate has varied from two weeks to over one year. Determinations of urinary calcium and phosphorus were obtained at frequent intervals on and off phytate, and occasional checks on serum values were obtained as well. In every instance there was a rapid

and marked reduction of urinary calcium of from 50–75% or from 129 to 345 mg./day varying with the dose administered, with return to pretreatment levels on cessation of therapy. We observed a concomitant rise of urinary phosphorus, which could be explained in large part by the additional phosphorus ingested with the phytate. No significant changes of either serum calcium, phosphorus or alkaline phosphatase were noted in the direction of osteomalacia, even after one year of treatment. Because of distressing side effects, including nausea, excessive gas and diarrhea, aluminum hydroxide gel was administered concurrently. Careful titration of these two agents made prolonged use feasible in the majority of patients. This combination seems even more rational, since it also tends to reduce the rise of urinary phosphorus observed while on phytate.

A Quantitative Comparison of the Response to Exogenous Thyrotropin in Euthyroid and Thyrotoxic Human Subjects

By *Monte A. Greer and Herbert F. Shull.* Radioisotope Service, V. A. Hospital, Long Beach, and the Department of Medicine, U.C.L.A. School of Medicine and University of Oregon Medical School.

Thyroidal secretion rates were determined in 20 euthyroid and 10 thyrotoxic patients by daily external scintillation counting following administration of 100–300 μC I^{131}. Reaccumulation of I^{131} liberated from the thyroid was blocked by daily administration of 90–180 mg. methimazole. Corollary studies were made of serum PBI^{127} and PBI^{131}. Following baseline studies, gradually increasing doses of intramuscular thyrotropin were given to both groups. The average daily dose at which an increased secretion rate was first seen in the euthyroid patients was 1.75 U.S.P. units, compared to 10 units for the thyrotoxic. In both groups stimulation of thyroidal secretion began well before 24 hours following administration of an effective dose of thyrotropin. A period of inhibition began 24 hours after the last thyrotropin injection. This was transient in 2 of the thyrotoxic patients and one of the euthyroid patients, but persisted for the remainder of the period of study in the other subjects. No qualitative difference in response could be detected between the two groups. The data, while inconclusive in themselves, are believed compatible with the hypothesis that thyrotoxicosis is due to an increased secretion of thyrotropin by the pituitary.

Evaluation of a New Oral Androgen in Neoplastic Diseases

By *John B. Field.* Department of Medicine, School of Medicine, University of Southern California and the Los Angeles County General Hospital.

In the attempt to alter the endocrine environment by administering androgens to female patients with inoperable and advanced tumors, present regimes have limitations. Testosterone must be injected two or three times weekly for long periods of time; the oral methyltestosterone (100–200 mg. daily) is unpredictable in its absorption and is expensive. A new oral agent, 9 α-fluoro-11 β-hydroxy-17αmethyltestosterone, known as fluoxymesterone (Halotestin), has been recently synthesized, and has an apparent oral androgenic activity of about 5–10 times that of methyltestosterone. It has been successfully utilized in male hypogonadism for periods of 4 to 6 months. This compound has now been evaluated in 16 patients with inoperable and, in many cases, advanced neoplastic diseases for periods up to 11 months. The most satisfactory results have been achieved in metastatic carcinoma of the breast. Of 7 such patients, to date 4 have had partial to full remissions from 3 to 11 months, at maintenance doses of from 20 to 30 mg. daily. With doses of from 5 to 15 mg. daily, some early recurrence or exacerbation was seen. A female of 77 years with many pulmonary metastases had slight improvement with testosterone (300 mg./week for 9 weeks), but fluoxymesterone 20 mg. daily induced a considerable subjective and objective remission for more than 5 months. A female of 40 years had complete resolution of numerous skin nodules within 3 weeks of a dose of 30 mg. daily. Two females with widespread advanced metastatic disease did not respond to fluoxymesterone or testosterone and succumbed within 2 to 5 months. One female of 40 years did not respond to oophorectomy or fluoxymesterone but is in a remission with corticosteroids. In three cases of inoperable carcinoma of the ovary with ascites treated with doses of 5 to 30 mg. daily, 2 patients have had partial remissions of 6 months in combination with other therapy; one was a therapeutic failure. Most females given a daily level above 10 or 15 mg. have evidenced signs of androgenicity, although this has been less than in those receiving 300 mg. parenteral testosterone weekly. There have been no other undesirable side effects; as with testosterone, some patients show increased hemoglobin levels.

Uric Acid Metabolism: The Oxidation of Uric Acid in Normal Subjects and Patients with Gout, Polycythemia and Leukemia

By *M. Pollycove, B. M. Tolbert, J. H. Lawrence and D. Harman.* Donner Laboratory and Donner Pavilion, University of California, Berkeley.

Uric acid is regarded as an end product of purine metabolism, requiring excretion for its disposal. Recently, other investigators have demon-

strated that urinary excretion comprises only about 85% of the daily removal of uric acid. This suggests that some uric acid may be broken down to other compounds or may be further degraded to furnish energy by oxidation. This study was undertaken to determine directly total body uric acid, its daily turnover, and the daily amount of uric acid oxidized to CO_2.

Two normal subjects and four patients with gout and/or polycythemia were studied and patients with leukemia are being studied. They were injected intravenously with 10–30 mg. of uric-2-C^{14} acid, specific activity 1–2.5 μc./mg. The specific activity and total amount of uric acid excreted in the urine, the fecal elimination of C^{14}, and the specific activity and total amount of CO_2 eliminated in the breath were determined following injection. $C^{14}O_2$ measurement was made continuously over representative periods, using an ionization chamber, vibrating reed electrometer, and CO_2 gas analyzer.

Normal subjects had total body pools of 650 and 1100 mg., daily turnover of 510 and 610 mg., urinary excretion of 440 and 510 mg., and oxidation of 55 mg. and 100 mg. uric acid. All the patients with gout and/or polycythemia had increased amounts of uric acid in the body. They also showed greater daily turnover, urinary excretion, and oxidation to CO_2 of uric acid. It was found in all cases that oxidation to CO_2 of the labeled carbon atom requires, on the average, several days.

The results of these measurements of $C^{14}O_2$ show that approximately 10–16% of uric acid formed is oxidized to CO_2, and that uric acid, therefore, is not entirely an end product of purine metabolism. In some patients whose body uric acid is increased, a greater percentage of uric acid is oxidized.

GASTROINTESTINAL SYSTEM

Further Studies on the Effect of Reserpine on Gastric Secretion and Its Possible Site of Action

By *J. Alfred Rider, Joyce Swader, John C. Gibbs, Jere Deroin, Lourdes A. Agcaoili and Hugo C. Moeller.* Gastrointestinal Clinic, Department of Medicine, University of California School of Medicine, San Francisco.

Reserpine has been used clinically with good results to treat a number of diseases or conditions in which anxiety states, tensions and aggressions play a prominent role either in symptomatology or etiology. Our previous studies have shown that a single dose of 0.5 mg. or more of Reserpine will increase volume and free acidity of gastric secretion. This effect increases with dose. A single dose of 0.25 mg. or less has no significant effect. Studies with oral Reserpine gave similar results. Pamine, an effective antisecretory agent, shows little if any blocking effect on the stimulated secretion produced by oral or parenteral Reserpine in amounts greater than 0.5 mg.

A study was made on patients who had no free acid in the basal gastric analysis following a vagotomy or a vagotomy and sympathectomy. As was expected, insulin did not stimulate the production of free HCl. Reserpine in a single parenteral 2.5 mg. dose caused the secretion of free acid; this was less than that obtained with the use of Histalog (analog of histamine).

These studies indicate that Reserpine may have a direct peripheral stimulating effect upon the gastric parietal cells.

Quantitative Analysis of the Radioactive Rose Bengal Test of Liver Function

By *Jerold M. Lowenstein.* Department of Medicine, Stanford University School of Medicine, San Francisco.

A quantitative method has been devised for interpreting the radioactive rose bengal uptake excretion test of liver function. When rose bengal tagged with I^{131} is given intravenously, the rise and fall of radioactivity in the liver can be measured with a gamma-ray detector. The shape of each "uptake excretion curve" is determined by three factors—the uptake and excretion rates (both exponential) and the liver blood pool. These quantities can be found by graphic analysis and are expressed as uptake halftime, excretion halftime and per cent of total blood volume in the liver.

In normal subjects, mean values were, respectively, 9 min., 1.5 hrs., and 18%; in Laennec's cirrhosis, 18 min., 5 hrs., and 32%; in biliary cirrhosis, 28 min., over 24 hrs., and 35%; in obstructive jaundice, 15 min., over 24 hrs., and 23%.

When there is hepatocellular damage, uptake is impaired, and obstruction causes delayed excretion. This analysis quantitates each of these factors and thus enhances the value of the radioactive rose bengal test in differential diagnosis of liver disease.

The Blood Disappearance of Radioactive Rose Bengal. A Rapid Simple Test of Liver Function

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The use of rose bengal for ascertaining liver function has been revived recently with the development of an I^{131}-labeled dye. Studies by Taplin and associates, and Brown and Glasser indicate the possible usefulness of radioactive rose bengal in the evaluation of liver disease. These workers have studied the liver uptake of radioactive rose bengal by direct liver counting. Inasmuch as the metabolic processing of rose bengal by the liver involves a number of complex hepatic functions, some of which may take place concomitantly, it was felt that a more discrete estimate of liver function could be obtained by studying the blood disappearance of the tracer.

An attempt has been made to develop a rapid, simple test utilizing a minimum of equipment and yielding clinically useful information concerning liver function. The disappearance of blood radioactivity following the injection of radioactive rose bengal has been estimated by peripheral scintillation counting, using standard thyroid I^{131} uptake equipment. External counting has been done over the lateral aspect of the head because of the large and stable vascularity of this area.

Preliminary studies on more than 50 patients reveal that the radioactive rose bengal blood disappearance is a good index of liver function. All studies have been correlated with the clinical condition of the patient and various liver function tests, including bromsulfalein tests, in every case. It appears that the rose bengal blood disappearance is, under some circumstances, more sensitive to minor alterations in liver function than tests presently available. The radioactive rose bengal blood disappearance has certain unique advantages in contrast to other presently employed tests of liver function. It is technically simple, rapid, requires no complex volumetric or colorimetric measurements, and is valid in the presence of jaundice, hemolysis and lipemia.

Hepatic Blood Flow, Oxygen Consumption and Wedge Pressure in Cirrhosis Before and After End-to-Side Portacaval Anastomosis

By *Allan G. Redeker, Herman M. Geller and Telfer B. Reynolds.* University of Southern California School of Medicine, Department of Medicine and Los Angeles County Hospital, Los Angeles.

Hepatic vein catheterization has been used to compare hepatic hemodynamics and oxygen consumption before and after construction of an end-to-side portacaval anastomosis in ten patients with cirrhosis and esophageal varices. Studies were made from a few hours to 55 days preoperatively and were repeated postoperatively as soon as hepatic bromsulphalein extraction would permit (within two months in eight cases, 3½ months in one and 8½ months in another). In nine patients hepatic blood flow, determined by the bromsulphalein infusion technic, decreased markedly (range 15.3% to 75.7%, mean 46.3%). In one, the flow increased slightly (16%). Wedged hepatic vein pressure fell approximately in proportion to the fall in blood flow (mean 38.5%). A value approximating hepatic vascular resistance was obtained by dividing wedged pressure by hepatic blood flow. This value did not change significantly following the shunt (mean values 0.0141 units preoperatively and 0.0170 units postoperatively).

The average value for hepatic oxygen consumption decreased slightly following the shunt (mean decrease 10.8%). The change in oxygen consumption was not a consistent one, increasing moderately in four patients and decreasing in six. The average arterial-hepatic venous oxygen difference preoperatively was 3.87 vol. % and 6.32 vol. % postoperatively, indicating a greater hepatic oxygen extraction with the decreased postoperative hepatic blood flow.

The fall in liver blood flow after portacaval anastomosis is presumably the result of deviation of portal blood into the vena cava. It can be inferred that the portal venous component of the total hepatic blood flow is still a significant one in cirrhosis, even with marked portal hypertension. In spite of the magnitude of this component, liver function at the time of the postoperative studies, as estimated by the usual biochemical tests and by the clinical course of the patients, was essentially unchanged from preoperative levels.

The Use of I^{131}-labeled Triolein in the Detection of Steatorrhea

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The subjects were given 100 microcuries of I^{131}-labeled triolein and 60 ml. of vegetable oil orally after an overnight fast. Blood samples were drawn 3, 6 and 9 hours later. A composite 72-hour stool sample was collected. Radioactivity of whole blood and of stool was measured and expressed as percent of dose administered. In ten patients without steatorrhea the peak blood level, which occurred most frequently in the 6 hour specimen, ranged from 1.03 to 4.02% of administered dose/L., and fecal excretion ranged from 0.35 to 4.26% of administered dose. In ten patients with subtotal

gastric resection the peak blood level was subnormal in only 1, whereas the fecal excretion was higher than normal in 5. In 3 patients with total pancreatectomy the peak blood level ranged from 0.11 to 0.53%/L. and the fecal excretion from 30 to 58% of administered dose.

INFECTIOUS DISEASES

Infections with Members of the Bacteriodes Fusiformis Group

By *Sydney M. Finegold and William L. Hewitt.* Department of Medicine, U.C.L.A. Medical Center, and the V.A. Center, Los Angeles.

The Bacteroides-Fusiformis group is made up of gram-negative, non-spore-forming anaerobic bacilli. Organisms are normally present in large numbers in the gastrointestinal tract, the mouth, and the female genital tract and are involved in a variety of infections, mostly originating in or near their normal habitats.

Chiefly within the last two years, we have observed 30 infections from which Bacteroides or Fusiformis were isolated. There were two bacteremias, three urinary tract infections, one conjunctivitis, three of peritonitis (two following ruptured appendix and one after a cecal perforation associated with carcinoma), five intra-abdominal abscesses (two appendiceal, two associated with carcinoma of the colon, and one tubo-ovarian abscess), ten other abscesses (including a brain abscess and a lung abscess with empyema), and there were seven wound infections (six of which were postsurgical). Only two of these 30 patients died and in neither of the fatal cases was the Bacteroides infection considered to be a major factor. Nevertheless, several of these infections were serious and ran a prolonged course. In three instances in this series, these infections were associated with malignancies of the bowel.

The Bacteroides and Fusiformis strains were recovered initially in Fluid Thioglycollate medium in all instances and subsequently were isolated in pure culture on blood agar plates (with or without neomycin added) incubated anaerobically. In 14 instances these anaerobes were isolated in pure culture; the other 16 strains were mixed with one or more of a variety of organisms, chiefly Lactobacillus, coliforms, and anaerobic or micro-aerophilic cocci. Antibiotic sensitivity tests on some of these strains and a number from other sources showed that tetracycline and chloromycetin were very effective in vitro and that erythromycin and, to a lesser extent, penicillin were effective against many strains. Therapeutic results achieved in this small group of patients were consistent with the in vitro sensitivity results.

Variations in Serum Glycoprotein Fractions in Pulmonary Tuberculosis

By *S. H. Lawrence, David Salkin and Henry E. Weimer.* San Fernando V. A. Hospital, and the Department of Medicine and Infectious Diseases, University of California, Los Angeles.

The glycoproteins of plasma are a heterogeneous group of conjugated proteins consisting of polysaccharides in firm union with peptides or proteins. Increased interest has been shown in this group of proteins in the past few years due to the demonstration that their concentration is markedly increased in a number of pathologic and physiologic states. With respect to infectious diseases, the carbohydrate-containing proteins of serum have been studied most extensively in relation to tuberculosis. Marked increases in the total serum glycoprotein and seromucoid have been reported in clinical tuberculosis and in experimental tuberculosis of guinea pigs and rabbits.

Method 10 of Cohen and associates, as adapted by Lever and co-workers, for the fractionation of five ml. amounts of human plasma into four primary fractions was used. It seemed possible that such a method might provide more information on the response of the carbohydrate-containing proteins to pulmonary tuberculosis than could be obtained from total plasma glycoprotein and seromucoid determinations.

Specimens from normal individuals and those with minimal, moderate, and far advanced tuberculosis in its active form and at various stages in its treatment were analyzed.

The upper limits of normal are hard to define but, in this paper, total glycoprotein values above 125 mg. and seromucoid above 14 mg. were considered abnormal.

The minimal cases showed normal values for total glycoprotein, seromucoid, and total protein. The moderate group had elevated glycoprotein and seromucoid in only 5% of the cases. The far advanced group showed increased total glycoprotein in 90% and increased seromucoid in 88% of the cases.

The distribution of the total protein showed marked changes only in the far advanced group.

Distribution of the glycoprotein had changes only in the far advanced cases with a percentage increase in Fraction VI and a decrease chiefly in Fraction I + III.

The greater the degree of exudation, the

greater the number of patients showing elevated glycoprotein and seromucoid values. By the same standards, the average levels were higher in the various groups.

The greater the extent of cavities, the higher the total glycoprotein and seromucoid values. This is presumed to be due to the greater amount of disease around larger cavities.

Patients with disease sufficiently extensive to cause marked elevation and whose clinical course was responsive to treatment showed gradual decline in all values. Patients with progressive fulminating disease showed continual elevation. The total polysaccharides dropped in 74% of those patients receiving medical therapy during 6 months hospitalization; the seromucoid dropped in 80%.

In patients who had resectional surgery with complete removal of the disease, 100% decreased in some category.

Levels in 10 terminal cases showed 90% elevation of total polysaccharides, 100% seromucoid, 60% gammaglobulin proteins and 100% in gammaglobulin polysaccharides.

The Effect of Para-aminobenzoic Acid on the Antimicrobially Active Serum Isoniazid Level

By *W. Mandel, W. F. Russell, Jr. and G. Middlebrook.* National Jewish Hospital, Denver, and the University of Colorado School of Medicine, Denver.

Isoniazid (INH) is metabolically altered to derivatives that possess little antimicrobial activity. The major pathway for this change is through acetylation. We have shown that para-aminosalicylic acid (PAS) increases the antimicrobially active serum INH level. Bell et al. have shown that in this regard para-aminobenzoic acid (PABA) has a similar effect. This study confirms and extends their observations.

The antimicrobially active serum INH level was determined by microbiologic assay. Blood was drawn for testing at 6 hours after a 4 mg./Kg. dose of INH in 12 adult tuberculous subjects and at 3 and 6 hours after the test dose in 5 subjects. The test was repeated under the same conditions with PAS and, at another time, with PABA. The dose of these drugs was either 5.0 Gm. given with the INH test dose or 2.5 Gm. given at this time and repeated 3 hours later.

On the addition of PABA, there was an increase in the antimicrobially active serum INH level in all 17 subjects. This increase was higher than that achieved by PAS in 10 subjects, identical with the increase in 4 subjects, and lower than the increase achieved by PAS in one subject. Studies with PAS were not performed in two subjects.

The rate of metabolic alteration of INH varies greatly in different subjects but is relatively constant for any one subject. Some subjects inactivate INH so rapidly that high dosages are insufficient to maintain desired serum levels of antimicrobially active drug. It is in these subjects that the concomitant administration of drugs such as PABA or PAS is indicated. PABA is more effective than PAS in increasing the antimicrobially active serum INH level. PABA or its metabolic derivatives presumably act by competing with INH for acetylation.

The Identification of the C-Reactive Protein and Various Antibodies in the Serum γ Globulin Obtained by Continuous Flow Paper Electrophoresis

By *R. J. Roantree, F. A. Pezold and Lowell A. Rantz.* Department of Medical Microbiology and Medicine, Stanford University School of Medicine, Stanford and San Francisco, California.

The technic of continuous flow filter paper electrophoresis was explored as a method for locating the electrophoretic fraction in which the C-reactive protein and several antibodies are found. Each electrophoretic run lasted 18 to 24 hours, using an early model of Durrum design. Constant current at about 6 ma and a voltage of 350–450 volts were employed. Both barbitol buffer pH 8.6 and phosphate buffer pH 7.2 were used. The latter gave better antibody yield. Ionic strength varied with the run from 0.006 to 0.012. The curtain was oven-dried and stained with Bromophenol Blue in the standard way. If the experiment was crucial, the curtain was air-dried and stained with Oil Red O, and then counterstained with Bromophenol Blue. In this way the boundary between the beta-lipoprotein and γ globulin could be demarcated. Fluid was collected from the drip points of the curtain and tested for the antibody or protein in question. Fractions collected, concentrated by dialysis and run on the standard Durrum hanging strip apparatus helped confirm the purity of the fractions.

Typhoid O, Salmonella group C, Brucella, heterophil and cold agglutins were located in the γ globulin. Antistreptolysin 0 and the C-reactive protein were also in this fraction.

The location of the C-reactive protein is the only questionable finding since it has previously been described as an alpha and a beta globulin and has been found in two cases of agammaglobulinemia. However, it was located in the γ fraction on 14 separate runs using sera from 8 patients with different diseases. On 3 occasions the fraction with the greatest concentrations of C-reactive protein corresponded to that with the greatest antistreptolysin concentration and, on one occasion, to that with the greatest concentration of Salmonella group C agglutinin. Hedlund has also recently found the C-reactive protein in the γ fraction.

KIDNEY AND BLADDER

Functional Hyposthenuria: A Reversible Tubular Defect Probably Secondary to Chronic Polydipsia

By *Charles R. Kleeman and Morton Maxwell.* Department of Medicine, University of California School of Medicine, and V. A. Center, Los Angeles.

Recent studies have demonstrated that the excretion of a continuously hypotonic urine (<150 mOsm./L.) during 3 days of sustained overhydration significantly impairs the ability to form a maximally concentrated urine after dehydration and pitressin. The present case appears to represent a clinical example of this phenomenon.

A 35-year-old male, with a 15-year history of polydipsia and polyuria, was admitted with a diagnosis of diabetes insipidus. Antidiuresis following dehydration, administration of pitressin, hypertonic salt, and nicotine indicated an intact neurohypophyseal system. However, urinary osmolarity did not *exceed* 300 msmO./L. A normal diuresis followed water ingestion: maximal rate and free water clearance (Ch_2O) 18 and 14 cc.'s respectively, minimals omolarity 40 mOsm./L.

Urinary sediment, culture, I.V.P.'s, retrogrades, and clearances of inulin and creatinine, and P.S.P. excretion were all normal. No calciuria, phosphoturia, amino aciduria, or potassium or sodium wastage existed. NH_3^+ and H^+ production were normal after NH_4Cl administration. Water ingestion continued and greatly exceeded needs.

After 3 weeks of enforced, mild, negative water balance a maximal urinary osmolarity of 650 mOsm./L. was attained during dehydration and pitressin infusion tests. This produced a markedly improved response.

It seems justified to conclude that this patient had an isolated functional defect in the "ultimate" concentrating segment of his renal tubule, that this defect was secondary to chronic polydipsia, and that temporary water restriction partially corrected this abnormality.

Arterial pH and the Regulation of Renal Acid Excretion

By *Daniel H. Simmons and N. S. Assali.* University of California Medical Center and the V. A. Center, Los Angeles.

Early studies have suggested that arterial pH and bicarbonate concentration were the main stimulus for renal acid excretion. Recently, it has been postulated inconclusively that arterial pCO_2 may also be a regulating factor and that arterial pH may not directly influence acid excretion. The present study was intended to assess systematically the role of arterial pH in the regulation of renal acid excretion. Studies were carried out on anesthetized female dogs in which minute ventilation was held constant with a respiration pump after giving a muscular relaxant. A steady state was maintained by monitoring expired gases and adjusting the ventilation rate to keep alveolar and arterial pCO_2 constant, thus allowing only plasma bicarbonate and arterial pH to vary. After three 30-minute control periods, infusions of 0.15 M HCl or $NaHCO_3$ were started at the rate of 0.3 cc./Kg./min. and collections were made for 3 additional periods. Changes in arterial pH, bicarbonate and pCO_2, as well as changes in urinary acid excretion (ammonia + titratable acid), were measured. The results show that during acid infusion, urine acid excretion increased, and vice versa. This established that arterial pCO_2 could not be the only regulatory blood factor, since, in these experiments, it remained constant. The rate of urinary acid excretion was then expressed as a function of arterial pH and plasma bicarbonate in a multiple regression equation. Statistical analysis of the regression coefficients indicated that arterial pH had no significant effect on renal acid excretion. Urinary changes in this experiment must therefore be ascribed to changes in plasma bicarbonate or filtered load of bicarbonate.

Renal Cortical and Medullary Electrolyte Changes Induced in Rabbits by Mercaptomerin Sodium

By *Jerry K. Aikawa.* Department of Medicine, University of Colorado School of Medicine, Denver.

Although the renal histopathologic effects of the organic mercurials are well-known, the nature of the changes in tissue composition is still obscure. Normal rabbits were injected intravenously with a single dose of 0.5, 1, 5, 10, 15 and 20 mg./Kg. of mercury as mercaptomerin sodium. The animals were killed by air embolism at 48 hours. The cortex was separated from the medulla by gross dissection. Aliquots of tissues were analyzed for water, sodium and potassium.

In uninjected control rabbits, the mean concentration of sodium in the medulla (115 mEq./Kg. wet wt. of tissue) was considerably higher than that found in the cortex (63 mEq./Kg.); no significant differences were noted in the mean potassium and water content.

The administration of mercury in doses of 0.5, 1 or 5 mg./Kg. produced no significant changes in the sodium, potassium or water contents of the renal cortex or medulla. 10, 15 or 20 mg./Kg. resulted in a significant increase in the cortical sodium concentration and water content and a decrease in the cortical potassium concentration. The medullary concentration of sodium was significantly and strikingly decreased following the injection of mercury in doses of 10, 15 or 20 mg./Kg.

The high concentration of sodium in the

medulla of uninjected control rabbits as compared with that in the cortex, in the absence of any significant difference in the potassium concentration or the water content, can be attributed to tubular fluid or to an intracellular accumulation. The data are compatible with the interpretation that renal-collecting duct cells may concentrate sodium. Doses of 10 mg./Kg. or more of mercury produced cortical tissue electrolyte changes compatible with cellular damage. In the medulla, these dosages caused a loss of the tissue's capacity to concentrate sodium, but no change in water and potassium.

Comparative Studies on the Effects of Mercurial Diuretics and Bed Rest on Renal Excretion of Water and Electrolytes

By *N. S. Assali, J. Voskian and J. Roller.* Department of Obstetrics & Gynecology, U.C.L.A. School of Medicine, Los Angeles.

The diuretic effects of oral and intramuscular organo-mercurials and of bed rest were studied in 29 patients with toxemia of pregnancy and 8 subjects with normal pregnancy. The patients were in the hospital and received the same daily diet containing 85 mEq. % Na as sodium chloride. They were studied during control, treatment and recovery periods, each period lasting from 3 to 5 days. Daily measurements of urine output, weight, plasma electrolytes, CO_2 and pH, as well as of urinary electrolytes, were made. The results show that the effects on excretion of water and electrolytes of oral mercurials in doses varying between 60 and 160 mg. of mercury were similar to those of bed rest. In contrast, intramuscularly administered mercurials produced a marked Na, Cl and, to a certain extent, K loss which was significantly higher than that produced by either oral mercurials or bed rest. Weight loss followed a trend similar to the changes in water and electrolyte excretion. No changes in acid excretion were encountered in any of the patients studied. It is concluded that in pregnant patients with or without toxemia of pregnancy, oral organo-mercurials, unlike the injectable form, do not have a striking diuretic effect and the slight diuresis observed in some patients might be attributed to bed rest. Impaired intestinal absorption of mercury may be the reason for the absence of diuretic effects of the oral compounds.

The Renal Clearance of Phosphate; The Influence of Blood Glucose and Serum Phosphate Levels

By *E. R. Huffman, C. J. Hlad, Jr. and H. Elrick.* Radioisotope Service and the Department of Medicine, V. A. Hospital, and the Department of Medicine, University of Colorado Medical School, Denver.

The renal clearance of both endogenous (C_{PO_4}) and exogenous labelled phosphate ($C_{P^{32}O_4}$) has been studied in 17 normal males. Utilizing standard renal clearance technics with creatinine as an indication of glomerular filtration, a sensitive and consistent parallel relationship between alterations in the serum glucose level and C_{PO_4} and $C_{P^{32}O_4}$ was observed. The $C_{P^{32}O_4}$ exceeded the C_{PO_4} from 30 to 86% in all but 2 subjects.

The rise in the C_{PO_4} occurred early after the infusion of glucose and was noted in 9 of the 13 subjects to occur in the first 10-minute period. By the third 10-minute clearance period, all subjects showed a definite increase. The correlation coefficient between blood glucose and phosphate clearance was significant ($P = .01$) in 11 of the 13 subjects studied; the mean coefficient for all experiments was 0.80. These coefficients were obtained from data resulting from both increasing and decreasing blood glucose levels. Peak C_{PO_4} values seldom exceeded 40 ml./min., and were obtained with serum glucose levels of 122 to 188 mg. %. Analysis of the data indicates that the increase in the C_{PO_4} and $C_{P^{32}O_4}$ is due to the decreased tubular reabsorption of phosphate brought about by the reabsorption of the increased load of filtered glucose.

Because of the differences in the C_{PO_4} and $C_{P^{32}O_4}$, observations were made after the infusion of a buffered phosphate solution with blood glucose remaining constant. Elevated phosphate clearances by this method far exceeded those resulting from hyperglycemia. Data obtained after increasing these clearances by phosphate infusion indicated that a portion of the endogenous plasma PO_4 is handled differently by the kidney than exogenous phosphate. Thus the "true" clearance of phosphate is represented by the labeled exogenous $P^{32}O_4$.

The Absorption of Sodium From the Human Bladder

By *Charles Vivion, C. J. Hlad, Jr. and B. Eiseman.* University of Colorado Medical Center and the V. A. Hospital, Denver.

This is a study of the possible role of sodium absorption from the urinary bladder in the development of the electrolyte imbalance that occasionally occurs following the relief of lower urinary tract obstruction. Previous studies from this laboratory have shown that sodium is absorbed from the bladder of dogs and that the rate of such absorption is markedly influence by the pH of the bladder fluid.

Bladder instillation of Na_{24} in physiologic saline has been carried out in 20 patients; absorption was determined by peripheral blood and bladder fluid studies for a 3-hour period following the injection of the fluid. Urinary contamination of the instilled fluid has been minimized by prior dehydration of the patient and by the administration of cortisone the day prior to the experiment.

Four groups of individuals have been so studied: (1) patients with normal bladders; (2) normals with

the instillate adjusted both to alkalinity and to acidity; (3) patients with chronically infected bladders; (4) paraplegics with chronically infected bladders.

Significant absorption of sodium was detected in only one individual in the entire series, a paraplegic with a diffuse chronic cystitis.

It is concluded that in man (contrary to the situation in the dog), the sodium absorption from the bladder is minimal and that alteration of the pH of the instilled fluid is ineffective in changing this response. It is further concluded that bladder absorption of sodium is not an important etiologic factor in the production of sodium and water imbalance following the relief of lower urinary tract obstruction.

NERVOUS SYSTEM AND MUSCLE

Azacyclonol (Frenquel) in the Treatment of Chronic Schizophrenia: A Double-Blind, Controlled Study

By *Theodore M. Odland.* Norwich, Connecticut.

The effect of Frenquel in chronic schizophrenia was evaluated by means of a special double-blind technic, which is presented as a step in standardizing the evaluation of psychopharmacologic agents.

The research team consisted of the researcher, three ward psychiatrists, a pharmacist and four nurse supervisors.

The patients were all residents on the continued treatment service of a large state mental hospital. They were divided into two equal groups on the basis of age, sex, duration of illness, duration of hospitalization, ideas of reference, delusions, hallucinations and behavior. Group I received the test drug for four weeks, followed by placebo for four weeks and a final four weeks of the test drug. Group II received placebo the first four weeks, test drug the second four weeks and placebo the third four weeks. Ward psychiatrists made written reports at the end of each four-week period.

In tabulating the data, 27 categories of response were utilized, based on the combinations and permutations of three different things taken three at a time.

Analysis of the data revealed that of the total number of 171 patients, 61 were improved in relation to the test drug and 11 became worse. The comparable figures for placebo were 28 and 8. In the other 63 patients, changes were minimal or unrelated to medication. Of the 61 improved in relation to the test drug, 37 were in Group I and 24 were in Group II.

Statistically, the difference in response to Frenquel and placebo is highly significant ($p = 0.0003$); this establishes the fact that Frenquel has a beneficial effect in schizophrenia. That a majority of improvement in relation to Frenquel occurred in Group I suggests that longer duration of therapy produces better results.

Adynamia Episodica Hereditaria

By *Jacques Crepeau, Loren T. DeWind and Robert R. Commons.* Hospital of the Good Samaritan, Los Angeles.

The syndrome of adynamia episodica hereditaria was first described by Gamstorp. This syndrome is to be distinguished from family periodic paralysis, muscular dystrophy and dystrophia myotonica. The case history of a patient believed to be afflicted with the disorder is presented.

A married woman, aged 35, resident of Kentucky, Idaho and California, presents with episodes of muscular weakness of 25 years' duration, becoming progressively worse after the age of 19, following a pregnancy. Attacks vary in severity from mild weakness, of a few minutes duration, to complete paralysis, lasting 24 to 36 hours. Average duration of attacks is 1 to 1½ hours. Attacks occur most frequently during rest following physical exercise. Exercise may abort attack. Between attacks there is no weakness. There is no relation between food intake and attacks. Family extraction is Scotch-Irish-Welsh; mother, maternal grandmother and great grandmother and father of great grandmother similarly afflicted. Three of five sons likewise afflicted. Three living brothers afflicted and some of their children. Physical examination remarkable only for absence of stigmata, muscular dystrophy, dystrophia myotonica and neurologic disorder.

Routine urinalysis, complete blood count normal; serology negative. Fasting blood sugar 112, serum calcium 10 mg. %, serum potassium 5.7 mEq. %, 4.3 mEq. %, 5.0 and 5.8 mEq. % during attacks. Protein-bound iodine 6.1 μg. %, cholesterol 334 mg. %. Electrocardiogram, and electroencephalogram normal. Electromyogram showed myotonic reaction and small motor units recorded in all muscles sampled bilaterally. Considered to be consistent with dystrophia myotonica. Three sons with disease similar to mother revealed same electromyograph findings.

Infusion of 50 Gm. glucose and 6 units of insulin

over three-hour period produced no subjective or objective weakness and revealed normal appearing glucose tolerance curve. Urinary 17-ketosteroid excretion 13.3 mg. in 24 hours. Therapy with potassium and methyl testosterone has produced no significant improvement.

RADIATION

Prolongation of the Normal Life Span by Radiation Protection Chemicals

By *Denham Harman*. Donner Laboratory and Donner Pavilion, University of California, Berkeley.

The theory has been advanced that aging may be due in part to the deleterious side effects of free radicals normally produced in metabolism. On the basis of this theory it would be anticipated that raising the concentration in the organism of compounds capable of reacting rapidly with free radicals would tend to slow down the aging process and thus lead to an extension of the normal life span. This possibility has been tested. Two short-lived strains of mice were employed, AKR (males) and C3H (females).

Thirty groups of mice were placed, shortly after weaning, on a powdered diet (ad lib.), to which was added a reducing agent. Each month the weight and number of survivors were recorded. No significant weight difference was noted between controls and those on effective chemicals. With rare exceptions the autopsied (no mice were autopsied in the first six months) dead AKR and C3H mice had lymphatic leukemia and large tumors (mammary carcinoma), respectively.

AKR mice on cysteine hydrochloride (1%), 2-mercaptoethylamine hydrochloride (1%), and 2,2′-diaminodiethyl disulfide dihydrochloride (0.5%) had a half life span (10 months) approximately 20% greater than the controls (8 months). The death rate of the controls on powdered diet was essentially the same as that of a group on pellets and that reported by the supply laboratory. At the age of 10 months the prolongation of life by these chemicals was significant at a P value of 0.01 or less.

These preliminary results are encouraging. The effect could easily have been missed as there was no way of determining beforehand just how much of the compounds should be added to the diet of any given strain to yield the desired result. Additional experiments are in progress.

The Binucleated Lymphocyte Response to Low-Level Radiation Exposure in Man

By *R. Lowry Dobson and Mary M. Chupp*. Donner Laboratory, Section on Research Medicine, University of California, Berkeley.

It now appears that physiologic effects of low doses of ionizing radiation in man may prove to be of significance. The use of radiation and radioactive materials in clinical medicine and research has increased during the past several years, reflecting a revolutionary growth in the production and availability of radioactivity. Consequent to large scale uses of radioactive materials, the radiation background of the earth's surface has risen perceptibly and continues to rise. The need for careful studies of the physiologic effects of small doses of radiation is apparent.

Although the hematologic effects of radiation have been well studied, changes in lymphocyte morphology and count have been the only effects described to follow low-level irradiation. Presented here are results of a quantitative study of the binucleated lymphocyte response in normal individuals chronically exposed to low doses of x- and gamma rays.

Binucleated lymphocytes were counted in the peripheral blood of 17 normal individuals. The examination of several million white cells on stained blood films was made possible by semi-automatic methods. A significant increase of binucleated lymphocytes in response to low-level radiation exposure was observed. The average incidence of these cells in a group of exposed persons was 5.9 per 50,000 leukocytes; the range was 3.4 to 7.6. The average incidence in a control group was 1.4; the range was 0.4 to 2.5. Studies carried out for periods of several months on individuals chronically exposed at near-maximum permissible levels showed that an elevated incidence of binucleated lymphocytes was maintained.

The binucleated lymphocyte response was seen in persons after single exposures to x- and gamma rays in the dose range of 100 to 300 mr.; it was seen in persons chronically exposed to levels considerably smaller than the presently accepted maximum permissible 300 mr./week.

RESPIRATORY SYSTEM

Lobarspirometry; Effect on Body Position

By *C. J. Martin, A. C. Young and John J. Koler.*

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Uniform oxygen and carbon dioxide concentrations in the alveolar air of normal man are dependent upon the same relationship between ventilation and blood flow existing throughout the lung. That this ratio of ventilation to perfusion (V/P) changes between an upper and lower lobe with changes in body position has been shown by lobar sampling of alveolar air. Higher oxygen and lower CO_2 concentrations are found in the upper lobe of erect man than in the lower lobe. In supine man these differences are erased or reversed. The present study sought to determine whether blood flow or ventilation changed with body position and, if so, to quantitate the change.

A lobar spirometric catheter has been developed in this laboratory. This three-lumen instrument provides a channel for the measurement of oxygen uptake and ventilation for the upper lobe and the lower lobe of one lung and the whole of the opposite lung. The lobar ventilation is directly measured and the oxygen uptake is a measure of the blood flow through that lobe. The following observations are made: (1) In changing from the supine to the upright position, the upper lobe oxygen consumption decreases by an average of 15% of the total uptake for the lung on that side. The lower lobe oxygen uptake increases by an equal amount. The distribution between the two lungs is unchanged by moving from the supine to the erect position. (2) The distribution of the minute ventilation between lobes and between lungs is essentially unchanged by moving from the supine to the erect position. (3) The distribution of the vital capacity between lobes or lungs is likewise unaffected by these positions.

Supine man has little difference between lobes in the V/P ratio. In erect man the V/P ratio in upper lobe is greater than that in lower lobe by a factor of almost two to one. This results from a relative decrease in blood flow through the upper lobe of erect man with a relative increase in flow through lower lobe and little or no change in ventilation.

The Inaccuracy of the Water-Filled Spirometer in the Measurement of the Maximum Breathing Capacity

By *William W. Stead, Herbert S. Wells, N. L. Gault and John Ognanovich.* V. A. Hospital, and the Department of Medicine, University of Minnesota, Minneapolis.

Bernstein has shown that the water-filled spirometer may record anywhere from 50–140% of the volume of air that is pumped into it during determination of maximum breathing capacity, depending upon the characteristics of the spirometer and the rate and depth of the respiration. We have studied both the 9 and the 13.5 L. Respirometers made by Collins and have obtained curves of frequency response that are similar in form to those given by Bernstein for the Knipping spirometer. The purpose of this paper is to illustrate some of the causes for the recording errors observed.

At rates of breathing and air flow that are involved in basal breathing, the spirometer records accurately (response of 100%). At frequencies of 80–90/min. the spirometer records less than the actual volume (response of 90%). At greater frequencies the spirometer overshoots (response progressively greater than 100%). The inaccuracy of the 9 L. spirometer is greater than that of the 13.5 L. spirometer.

Analysis of the physical reasons for the discrepancies has revealed the following: (1) The early under-response is due to the motion of the water as it is forced up and down by the rapidly changing pressures. This effect can be lessened considerably by the use of a light plastic bell which fits close to the inner drum of the spirometer. (2) The later and progressively greater overshoot of the recording pen is due to a combination of factors, the greatest of which are the inertia associated with the weight of the metal bell and its counter-weight and the snapping and slacking of the chain that connects the moving bell with the recording pen. These defects were improved by mounting the writing pen directly on the light plastic bell. We are presently working on other causes of inaccuracy in spirometer recording, and on a design for a better instrument.

Peak Oxygen Uptake of Healthy Young Men as Determined by a Treadmill Method

By *N. Balfour Slonim.* Division of Industrial Medicine, University of Colorado Medical Center, Denver.

The peak oxygen uptake of 50 healthy young white men (naval aviation cadets) was determined by a treadmill method. Each subject had undergone at least five weeks of intensive physical training. The treadmill speed was held constant at 3.5 mph and the tests at each treadmill grade were of 6 minutes' duration. Subjects were tested successively at 20%, 24%, 26%, and 28% grade until failure to complete a test. The grade was then decreased in steps of 1% until a test was found which could be completed. Motivation of the subjects was considered to be exceptional. Environmental temperature and humidity were closely controlled. Peak oxygen uptake is defined arbitrarily for the purpose of this study as the highest value obtained for rate of oxygen uptake as determined by measurement and analysis of expired gas collected during the 6th

minute of exercise. The mean peak oxygen uptake was found to be 4.05 L./min. with a standard deviation of 0.39 and a range of 3.22 to 5.17. The mean expiratory minute volume was found to be 147 L./min. with a standard deviation of 20, and a range of 95.2 to 201. These values exceed those generally accepted as occurring during muscular work. The mean expiratory minute volume was 83% of the mean maximal breathing capacity in 33 subjects in whom the latter was determined.

Cardiopulmonary Studies in Anoxia and Polycythemia Associated with Obesity

By *Jerome E. Cohn, I. T. Kurita, Ramon Lange, and Hans H. Hecht.* Pulmonary Disease Service, V. A. Hospital, Fort Douglas Division, and the Department of Medicine, University of Utah College of Medicine, Salt Lake City.

A syndrome of excessive obesity, drowsiness, arterial desaturation, and polycythemia, occurring in the absence of intracardiac or intrapulmonary shunts, has recently been described. The present report deals with two patients exhibiting these features on whom detailed cardio-respiratory studies were performed. Patient no. 1 was studied during one year of weight reduction, and Patient no. 2 during a 6-month weight gain.

Patient 1 (weight 355) initially had pulmonary hypoventilation (arterial CO_2 partial pressure [$Paco_2$] = 50 mm. Hg), arterial desaturation, and abnormal lung volume subdivisions. Pulmonary artery (PA) pressure was elevated. Volume of packed red cells (VPRC) was 56%, and right bundle branch block (RBBB) was present. After 80-pound weight loss normal values were obtained for PA pressure, $Paco_2$, lung volumes, and maximal oxygen diffusing capacity. VPRC had declined to 45%, but slight arterial desaturation persisted (SO_2 = 89%).

Initially, patient 2 (weight 225) was moderately obese and showed slight arterial desaturation and abnormal lung volume measurements. As he gained 40 pounds, these values worsened and elevation of $Paco_2$ and VPRC developed. Final measurements were comparable to the initial values observed in patient 1.

The most pronounced lung volume abnormality was low expiratory reserve volume (ERV) present initially in patient 1 and, finally, in patient 2. These low volumes made residual volume/total lung capacity (RV/TC) ratios abnormally high; large, poorly ventilated spaces resulted, leading to arterial desaturation, secondary polycythemia, and pulmonary hypertension. Continued alveolar hypoventilation caused hypercapnea.

With weight loss the ERV and RA/TC returned to normal in patient 1. Except for mild arterial desaturation and persistent RBBB, other measurements also became normal.

Most investigations of obesity emphasize cardiovascular complications, especially hypertension and arteriosclerosis. The data presented indicate another system which may be impaired by extreme obesity.

RHEUMATIC STATES

Steroid Therapy of Rheumatic Fever

By *Matthew L. Gibson, Jr.* Department of Pediatrics, University of Colorado, Denver.

Since July 1, 1954, 78 children with acute rheumatic fever, according to the diagnostic criteria of Jones, were treated with cortisone orally in a dosage of approximately 3 mg. per pound of body weight daily. They were maintained on this dose until all clinical and laboratory evidence of disease activity had disappeared. Therapy was controlled by the weekly determination of serum mucoprotein levels.

Random cases were also treated with ascorbic acid, 500 mg. daily. All patients were treated with parenteral penicillin on admission and were maintained on oral penicillin throughout their hospital stay. All patients received 2–3 Gm. potassium chloride daily.

Beginning in February, 1956, bed rest was abandoned as part of the management and the children were permitted to be up ad lib.

In patients with pre-existing heart disease, the course was not altered by the regime employed. Neither early ambulation nor the administration of ascorbic acid had any effect on the course of the disease. None of the 47 consecutive cases of acute rheumatic fever has to date any residual heart disease and all had lost their murmurs by the time of discharge from the hospital.

The Absorption of Heavy Water and Radioactive Sodium from the Knee Joint of Normal Subjects and Patients with Rheumatoid Arthritis

By *Philip R. Lee, John F. Scholer and Howard F. Polley.* Mayo Foundation and Mayo Clinic, Rochester, Minnesota.

This study was done to compare the rate of absorption of water and sodium from the synovial

space of the knee in both normal subjects and patients with rheumatoid arthritis, and in the latter, to determine the effect of intra-articular hydrocortisone on these rates. We know of no previous studies in which this comparison has been made.

The method used was that of Scholer and Code. In this method, measurement of the arterial blood concentration of the isotope is used to obtain the rate at which the isotope enters the blood from the space in question.

Two normal and five rheumatoid arthritic subjects were studied. In three of the latter, studies were done before and after intra-articular Compound F injection. The results demonstrated that sodium and water disappear from the synovial cavity of the knee at a constant rate. Water disappears at a rate which is slightly faster than sodium in normal subjects, patients with rheumatoid arthritis, and after the intra-articular injection of hydrocortisone. The rate of absorption of both heavy water and radio-sodium was decreased after the injection of hydrocortisone.

Conclusions: (1) The method of Scholer and Code can be applied to the study of heavy water and radio-sodium absorption from the synovial space; (2) the results obtained by others for the absorption of radio-sodium from the normal joints are confirmed; (3) the changes occurring in patients with rheumatoid arthritis and after the injection of Compound F are of a general nature and affect sodium and water absorption from the knee joint similarly.

PROGRAM

WESTERN SOCIETY FOR CLINICAL RESEARCH

Thursday, Friday and Saturday, January 31, February 1 and 2, 1957

The Golden Bough Theater, Carmel, California

Dr. William M. M. Kirby, Presiding

THURSDAY, JANUARY 31, 1957

1:45 p.m.

1. **Preliminary Observations on the Use of Nitrogen Mustard in Disseminated Coccidioidomycosis.**
   N. B. Kurnick, Long Beach.
   page 79
2. **Anti-Inflammatory Properties of a Pyrimidine Derivative, RO 2-5383/2.**
   Cutting B. Favour, Palo Alto.
   page 86
3. **The Variability of the Enteric Bacilli in Their Sensitivity to the Bactericidal Activity of Serum from Normal Subjects and Patients with Various Diseases.**
   R. J. Roantree and L. A. Rantz, Stanford.
   page 79
4. **Effect of Protein Feeding on Serum Enzyme Levels in Patients with Metastatic Carcinoma.**
   Laurens P. White, San Francisco.
   page 84
5. **Studies on the Alveolar-Arterial Oxygen Gradient in Acute and Chronic Poliomyelitis.**
   Stanley N. Rokaw, John E. Affeldt, Clarence R. Collier,* Milton G. Crane* and Andrew F. Farr,** Hondo.
   page 85
6. **The Effect of Mobilization on Hypercalciuria Following Acute Poliomyelitis.**
   Fred Plum and Marcelle F. Dunning, Seattle.
   page 73
7. **Competitive Inhibition of Mammalian Tyrosinase by Phenylalanine and Its Relationship to Hair Pigmentation in Phenylketonuria.**
   Masamitsu Miyamoto and Thomas B. Fitzpatrick*, Portland.
   page 86
8. **The Effects of Variations in Potassium Concentration on Ion Transport and Bioelectric Potentials Across the Frog Gastric Mucosa.**
   John B. Harris, H. Frank* and I. S. Edelman*, San Francisco.
   page 75
9. **The Influence of Certain Osmotically Active Substances on the Volume of Dog Kidneys.**
   C. A. Nugent and F. H. Tyler*, Salt Lake City.
   page 81
10. **Periodic Paralysis Associated with Hyperthyroidism and the Role of Aldosterone in the Pathogenesis.**
    *Milton G. Crane,** Los Angeles. (Introduced by *John E. Peterson.*)
    page 69
11. **Acute Effects of 9αFluorohydrocortisone and 2-Methyl-9αFluorohydrocortisone on Glomerular Filtration and Renal Clearances of Sodium and Potassium in Normal Subjects.**
    Jonas H. Sirota, Marcus A. Krupp, Bernard J. Axelrad, G. James Tobias* and Jean Fellows,** Palo Alto.
    page 80
12. **Factors Influencing Variance of Blood Volume in Normal Men.**
    E. Brown, J. L. Hodges, Jr., R. Wenneslland,* J. Hopper, Jr., K. G. Scott,* I. N. Tucker,* O. Guttentag* and B. Bradley,** Berkeley and San Francisco.
    page 53
13. **The Labeling of Serum Albumin for Turnover Studies.**
    Sheldon Margen and Harold Tarver, Berkeley.
    page 73
14. **Significance of Macroglobulins.**
    Arno G. Motulsky, Nils Eriksen, Wade Volwiler and Dennis Donohue,** Seattle.
    page 58

FRIDAY, FEBRUARY 1, 1957

9:00 a.m.

Dr. S. Gilbert Blount, Presiding

15. **Study of the Essential Amino Acid Requirements of Men over Fifty.**
    Stewart G. Tuttle, Marian E. Swendseid,* Wendell H. Griffith* and Samuel H. Bassett*, Los Angeles.
    page 74

*By invitation

16. **Blood Amino Acid Patterns in Hepatic Coma.**
Sherman M. Mellinkoff, Marjorie Frankland, Margaret Greipel* and Henry Shibata,** Los Angeles.
page 77

17. **"Direct" Bilirubin Production in Rat Tissue Homogenates.**
G. M. Grodsky and J. V. Carbone,** San Francisco. (Introduced by *V. M. Sborov.*)
page 78

18. **Catheterization of the Portal Vein Through a Portacaval Anastomosis in Patients with Cirrhosis.**
Telfer B. Reynolds, Herman M. Geller and Alan G. Redeker,** Los Angeles.
page 76

19. **Simultaneous Recording of (I^{131}) Transients from Four Crystal Detectors on the Anterior Thoracic Wall.**
Wayne Crockett, Daniel Parrish* and Rex L. Huff*, Seattle.
page 60

20. **Evaluation of Cardiac Reserve.**
Robert A. Bruce, Theodore J. Fuller and William W. Andrus,* Curt A. Wiederhielm and Charlotte Hamilton*, Seattle.
page 63

21. **Stenosis of a Branch of the Pulmonary Artery.**
Frederic L. Eldridge, Arthur Selzer and Herbert N. Hultgren, San Francisco.
page 61

22. **Paroxysmal Rapid Ventricular Response in Patients with Atrial Fibrillation as a Paradoxical Manifestation of Digitalis Intoxication. Report of Four Cases Successfully Treated with Intravenous Potassium.**
*Marvin B. Bacaner,** San Francisco. (Introduced by *Frederic Eldridge.*)
page 63

23. **Hemodynamic Patterns in Clinically Controlled Left Ventricular Failure.**
*Arthur Selzer and Donald J. McCaughey,** San Francisco.
page 64

24. **Simultaneous Human SGO-T, SGP-T and SLDM in Doubtful or Complicated Myocardial Infarction.**
J. J. Sampson, H. Weisberg, A. Lieberthal* and A. E. Lewis,** San Francisco.
page 64

25. **Masked Mitral Stenosis: The Pulmonary Hypertensive Heart and Mitral Insufficiency.**
Ramon L. Lange, R. Lawrence Sifford* and Hans H. Hecht*, Salt Lake City.
page 61

26. **Transthoracic Catheterization.**
*Hans H. Hecht and Ramon L. Lange,** Salt Lake City.
page 60

12:00 noon

BUSINESS MEETING

SATURDAY, FEBRUARY 2, 1957

9:00 a.m.

Dr. Daniel H. Labby, Presiding

27. **Arginase Activity of Erythrocytes and Leukocytes with Particular Reference to Pernicious Anemia and Thalassemia.**
John Reynolds, James H. Follette and William N. Valentine*, Los Angeles.
page 54

28. **The Interference of Unusual Plasma Proteins with the Clotting Mechanism.**
*Henry H. Henstell and Miriam Feinstein,** Los Angeles.
page 58

29. **Immunologic and Serum Protein Studies in Idiopathic Neutropenias.**
Charles Brubaker and Phillip Sturgeon*, Los Angeles.
page 55

30. **Hereditary Nonspherocytic Hemolytic Anemia in an Asymptomatic Family—A Clinical, Erythrokinetic, and Biochemical Study.**
*Irwin M. Weinstein and Roy Walford,** Los Angeles.
page 54

31. **Diisopropylfluorophosphate^{32} (DFP^{32}) as a Leukocyte Label.**
J. W. Athens, A. M. Mauer,* Helen Ashenbrucker,* G. E. Cartwright and M. M. Wintrobe*, Salt Lake City.
page 56

32. **The Effect of Reserpine on Pituitary-Adrenal Function.**
John A. Anderson, Minneapolis.
page 68

33. **A Study of Insulin Metabolism Using Insulin-I^{131}.**
Arne N. Wick and Douglas R. Drury, La Jolla and Los Angeles.
page 68

34. **The Concentration and Binding of Thyroxine and Triiodothyronine by Rat Diaphragm.**
John R. Hogness, Norman D. Lee, Margaret Berg* and Robert H. Williams*, Seattle.
page 66

35. **In Vitro Stimulation of Rat Adrenocortical Secretion by Corticotropin and a Protein Factor from Human Urine.**
Patrick J. Mulrow, Amos H. Lieberman,* George L. Shmagranoff* and John A. Luetscher, Jr.*, San Francisco.
page 69

SATURDAY, FEBRUARY 2, 1957

ALTERNATE SESSION

9:00 a.m.

Dr. Donald W. Petit, Presiding

Advance Reports Submitted to the Tenth Annual Meeting of the

Western Society for Clinical Research

The Golden Bough Theater, Carmel, California

Thursday, Friday, and Saturday, January 31, February 1 and 2, 1957

BLOOD

Factors Influencing Variance of Blood Volume in Normal Men

By *E. Brown, J. L. Hodges, Jr., R. Wennesland, J. Hopper, Jr., K. G. Scott, I. N. Tucker, O. Guttentag and B. Bradley.* Department of Medicine, University of California School of Medicine, San Fancisco, the Department of Statistics, University of California, Berkeley, and the Radioactivity Research Center, University of California, San Francisco.

Blood volume was measured with Cr^{51} in 201 healthy male prison inmates in an effort to improve means of predicting normal values. Variability is reduced to an important degree when red-cell or plasma volumes are related to height and weight combined, rather than to either measurement alone. (S.D. about the regression line of red-cell volume related to height = 0.248 L., related to weight = 0.197 L., and S.D. about the regression *plane* of red-cell volume related to height and weight combined = 0.191 L.). The equations for the 3-dimensional regressions of cell or plasma volumes against height and weight combined can be illustrated graphically by a chart in which height and weight are plotted on a plane surface and the intersections of planes representing mean predicted volumes are shown as lines. Use of such a diagram has advantages over the conventional regression of volumes against surface area. Individual deviations from the usual weight : height relationships are revealed strikingly, whereas they are concealed when surface area is calculated according to Du Bois' formula. Illustrative examples from healthy and pathologic material will be shown. Analysis of residuals in the 3-dimensional regression system shows that consideration of somatotype improves prediction only slightly. Subjects accustomed to heavy physical work tend to have larger volumes than sedentary ones. Plasma volume is relatively more dependent on height, and red-cell volume on weight; tall, thin subjects tend to have lower hematocrits than short, heavy ones. The volume of blood per unit body size appears to be the same in large and small subjects, whether related to weight, to height, to surface area, or to height and weight combined.

The Corpuscular Constants in Infancy, Their Relation to Iron Nutrition

By *Phillip Sturgeon.* Hematology Research Laboratories, Childrens Hospital, Los Angeles.

Compared to standards for normal adult males, most hematologic surveys of normal infant populations show reduced hemoglobin, MCV and MCHC. Measurements have been made on 93 twelve-month-old normal infants (range, 11 to 14 months of age) and 28 normal infants given large parenteral doses of iron. Both groups come from upper economic segments of the population. In addition to the cytologic studies, measurements of serum iron, iron binding capacity, copper and erythrocyte protoporphyrin were made.

With respect to the mean cell volume these studies indicate (1) under optimum conditions of iron nutrition, the normal one-year-old infant's mean cell volume is on an average 79μ^3 (range, 70 to 84μ^3); (2) mean cell volumes in the lower normal range (70 to 74μ^3), although generally associated with additional signs of iron deficiency, do not necessarily signify iron deficient states; (3) infants with mean cell volumes above the normal average

may nevertheless have signs of iron deficiency; (4) frankly anemic infants may have mean cell volumes well within the normal range; (5) the average erythrocyte microcytosis and wide range of values common to this age represents a physiologic variable related to age that can be influenced, but slightly, through supplemental iron nutrition.

With respect to the mean erythrocyte hemoglobin concentration, these studies indicate (1) a more definite direct relation to iron nutrition; under optimum conditions this value is on an average 31% (range 30 to 33%); (2) for the most part infants having values of 29% or less have numerous associated findings indicative of iron deficiency; (3) those with values of 32% or more have very few findings indicative of iron deficiency, irrespective of their MCV; (4) frankly anemic infants have values of 28% or less; (5) the minimal value observed in infants given large supplements of iron was 30%.

Hereditary Nonspherocytic Hemolytic Anemia in an Asymptomatic Family—A Clinical, Erythrokinetic and Biochemical Study

By *Irwin M. Weinstein and Roy Walford.* Departments of Medicine and Pathology, University of California Medical Center, Los Angeles; and Sawtelle V. A. Center, Los Angeles.

A study of nonspherocytic hemolytic anemia occurring in an asymptomatic family revealed markedly abnormal autohemolysis by in vitro tests, severe derangement of erythrocyte phosphate partition, and elevated red cell porphyrins. This family differed clinically and biochemically from reported cases of both hereditary spherocytic and nonspherocytic disease.

Methods included osmotic fragility studies, Selwyn-Dacie test for autohemolysis, enzymic determination of adenine nucleotides in the erythrocyte, erythrocyte and urinary porphyrin analyses, and measurement of erythrokinetics with radiochromium and radio-iron tracers.

Data were obtained on a 24-year-old white married female, her two children, and her mother, all of whom were clinically well. A slight anemia and reticulocytosis were present in the patient and her mother. Splenomegaly was detected only in the mother. Erythrocyte morphology on peripheral blood smear and osmotic fragility of washed erythrocytes initially and after 24 hours incubation were normal in all instances. The Selwyn-Dacie test for autohemolysis after 24 and 48 hours of incubation was markedly abnormal in all of the above members of the family. This was not corrected by addition of glucose and/or adenosine. Determination of ATP, ADP, and AMP in the patient's erythrocytes revealed values within normal limits for ADP and AMP but almost zero values for ATP. The patient's red cell uroporphyrin and coproporphyrin levels were elevated. Weakly positive Watson-Schwartz reactions were intermittently present in these family members; but no urinary uroporphyrin or coproporphyrin could be demonstrated. Combined Cr^{51} and Fe^{59} tracer studies of the patient's blood confirmed the presence of a hemolytic process, including sequestration of red cells in her spleen.

The fundamental biochemical abnormalities of erythrocyte metabolism observed in this family differ, at least in degree, from those reported for hereditary spherocytosis and for the red cell storage-lesion. It is of interest that biochemical derangements of this severity may exist without significant clinical manifestations.

Arginase Activity of Erythrocytes and Leukocytes with Particular Reference to Pernicious Anemia and Thalassemia

By *John Reynolds, James H. Follette and William N. Valentine.* Department of Medicine, School of Medicine, University of California Medical Center, and the V. A. Center, Los Angeles. (Aided by grants from U.S.P.H.S., Parke Davis and Co. and Gladys F. Bowyer Fund.)

The activity of arginase in human erythrocytes and leukocytes was assayed in normal subjects and in various diseases. Blood corpuscles were first separated by enhanced sedimentation in 4% bovine fibrinogen solution. Saline-washed leukocytes or laked erythrocytes were incubated for six hours at 37° C. with a buffered 1-(+)-arginine substrate. The urea so formed was converted to ammonia by adding urease, and final determinations were made spectrophotometrically after Nesslerization. Arginase values were expressed as mg. of urea nitrogen per 10^{11} RBC and per 10^{10} WBC.

The mean unit cell arginase activity of normal leukocytes was 50 times that of erythrocytes. The mean value for erythrocyte arginase in normals was 21.0, and a range of 0 to 46.2 was observed. Fourteen patients with pernicious anemia and two with other nutritional megaloblastic anemias showed a range of 50.2 to 167. The mean of 105.4 for this group was five times normal. As hematologic improvement occurred, the initially high erythrocyte arginase activity gradually returned to normal levels in all eight cases that were studied serially. A similar consistently high value was found in five patients with thalassemia major. No uniform deviation from normal was found in any of several other types of anemia, including those associated with malignant leukopathies, iron deficiency, hemolysis, infection, azotemia, and hemoglobinopathies.

Arginase activity was found to be present in normal and diseased white cells. The only uniform aberration from normal was found in chronic lymphocytic leukemia and acute blastic leukemia, suggesting that mononuclear cells have little arginase activity.

The role of arginase in human erythrocytes and leukocytes remains unknown. Likewise, there is no proven explanation for the uniformly high erythrocyte arginase activities found thus far only in un-

treated megaloblastic anemias and thalassemia major.

A Study of the Incidence of Blood Group Antibodies: a Hazard in Blood Transfusion

By *Eloise R. Giblett.* King County Central Blood Bank and the Department of Medicine, University of Washington School of Medicine, Seattle.

The use of blood transfusion in medical practice has steadily increased during the past 15 years. During that period, numerous blood group antigens capable of producing significant antibody response have been described. These antibodies constitute a definite threat as a cause of transfusion reactions and hemolytic disease of the newborn. Many of them can be detected only with such special technics as the indirect antiglobulin test.

In order to obtain data concerning the number of sensitized people in the area serviced by the King County Central Blood Bank, a screening technic was utilized. For a one-year period, the sera of all pregnant women submitted to this laboratory as well as the sera from all donors with a history of transfusion or pregnancy were examined for the presence of antibodies outside of the ABO system. In addition, all patients given blood transfusions over a six-month period were similarly screened. This study was carried out using the antiglobulin test and a panel of red cells of known antigenicity.

From a total of 34,789 donors, 2038 gave a history of pregnancy and/or transfusion. Of this group, 48 (or 2.3%) were found to have antibodies. Of 7650 recipients of blood (whose previous history was usually not available), 107 (or 1.4%) were found to be sensitized at the time the crossmatch was performed. Of 6453 maternal sera, 276 (or 4.3%) had positive screens. In this group, 10% of the Rh negative women had antibodies, whereas only 0.6% of the Rh positive women were sensitized. However, in the latter group, six mothers gave birth to babies with hemolytic disease of the newborn due to anti-c, anti-E or anti-Kell.

The antibodies in the donor and recipient sera showed an unexpectedly high incidence of anti-Kell and anti-Le^a. Both antibodies are capable of significant red cell destruction; thus their detection in crossmatching is important.

The results of these studies indicate the necessity for utilizing sensitive tests, preferably including the antiglobulin technic, in determining blood group compatibility. Furthermore, one of the important hazards in performing a blood transfusion, especially in the absence of definite indication, is emphasized.

A Serologic Study of Anti-Leukocyte Antibodies in Pathologic Sera

By *Roy L. Walford.* Department of Pathology, University of California School of Medicine, Los Angeles. (Aided by a grant from the Blood Bank of San Bernardino-Riverside Counties.)

Sera of rabbits immunized with lyophilized human white blood cells and the sera of three patients giving a strongly positive leukoagglutinin test were used in an investigation of leukoagglutinins, leukoprecipitins, agglutination of antigen-coated tanned sheep erythrocytes, and the effect of anti-leukocyte antibodies upon leukocyte amoeboid motility. The patients had hematologic diseases and had received multiple transfusions. Their sera demonstrated leukoagglutinins in a titer of 1 to 4 to 1 to 16; and showed significant inhibitory effect upon amoeboid motility after prolonged (18-hour) incubation with viable white blood cells, as judged by phase-contrast microscopy. No inhibitory effect could be demonstrated with 1 to 4 hours of incubation. Control sera showed no significant inhibitory action upon leukocyte amoeboid motility after either 4 or 18 hours of incubation. Leukoprecipitins and antibodies for antigen-coated tanned sheep erythrocytes were absent or not demonstrated in the human sera, but were present in immune rabbit sera. By precipitation in an agar medium, two separate antigens were demonstrated in lyophilized white cell mass by use of immune rabbit sera.

The role of anti-leukocyte antibodies in the manifestations of certain hematologic diseases may be fundamental, and has received emphasis in recent literature. Of equal interest is the possibility that the development of homospecific antileukocyte antibodies may represent a variety of the so-called homograft reaction, one that is amenable to in vitro serologic study. A delayed cytotoxic effect, manifested in this instance in the amoeboid motility studies, may be of basic importance in the homograft reaction.

Immunologic and Serum Protein Studies in Idiopathic Neutropenias

By *Charles Brubaker and Phillip Sturgeon.* From the Hematology Research Laboratories, Childrens Hospital of Los Angeles.

Using recently developed, clinically applicable immunologic and electrophoretic technics in conjunction with standard blood and bone marrow morphologic methods, a classification of idiopathic neutropenia having improved prognostic and therapeutic implications has been attempted.

Filter paper electrophoretic studies of the serum proteins were done using the Spinco Model R apparatus and the recently revised Spinco procedure. Inhibition of the antiglobulin (Coombs) test, a sensitive measure for globulin content of serum, was assayed by the method of Wiener, Hyman and Handman. Blood group isoantibody titer of the serum, as well as tests for specific immune reactions, were also carried out.

The results of the above procedures indicate that some cases of idiopathic neutropenia are associated with hypogammaglobulinemia and evidences of impairment of immune mechanisms.

Other cases of neutropenia do not have the serologic defect. Therapeutically, the administration of gamma globulin has proved beneficial in the management of the serologically deficient patients.

Diisopropylfluorophosphate^{32} (DFP^{32}) as a Leukocyte Label

By *J. W. Athens, A. M. Mauer, Helen Ashenbrucker, G. E. Cartwright and M. M. Wintrobe.* University of Utah, Salt Lake City.

Measurement of leukocyte "survival time in the blood stream" with adenine C^{14} or radiophosphate is impractical because the purification and measurement of the labeled nucleic acid is tedious and complex, and requires a large volume of blood. We have found that DFP labeled with P^{32} (DFP^{32}) is bound to leukocytes. The present study suggests a clinically applicable method based on this observation.

Two mg. of DFP^{32} are administered intramuscularly. A leukocyte-rich suspension is obtained and the activity per mg. of leukocyte nitrogen determined.

Since erythrocytes label to a greater extent than leukocytes, leukocytes must be obtained free of erythrocyte contamination. This has been accomplished by two means. First, partial saturation of the large erythrocyte pool with unlabeled DFP given one hour before the DFP^{32} resulted in a 5- to 10-fold reduction in erythrocyte activity, while leukocyte activity was increased 2 to 7 times. Second, a two-step separation technic (dextran sedimentation followed by gramicidin-lysolecithin hemolysis) removes 99.6 to 100% of the red cells. These two procedures reduce contamination from erythrocytes to less than 4.5% of total activity present in the leukocyte fraction. Different decremental slopes for leukocyte and erythrocyte radioactivity have been demonstrated; also, high erythrocyte activities persist 25 days after injection, while insignificant leukocyte activity remains. Thus, the measured leukocyte activity is independent of erythrocyte activity.

DFP^{32} is also bound to platelets which may contaminate the leukocyte fraction. However, the activity in platelets, as compared to leukocyte activity, is small and the method used for separation of white cells effectively eliminates platelets.

Customary methods for counting P^{32} proved unsatisfactory because of low sensitivity. Therefore, a plastic scintillation detector with 4-π geometry has been developed. With this method, counting rates are increased 2- to 5-fold as compared with end-window Geiger counting.

The Function of Hexokinase and Adenosinetriphosphatase in Controlling the Glycolytic Rate of Normal and Leukemic Leukocytes

By *William S. Beck.* Atomic Energy Project, University of California Medical School, Los Angeles.

As previously reported, marked differences are observed in the aerobic glycolytic rates of normal (N), chronic myelocytic leukemia (CML), and chronic lymphocytic leukemia (CLL) leukocytes, whose rates of lactic acid production from glucose are 30.1, 12.7, and 4.8 μmoles per hour per 10^8 cells, respectively.

An attempt was made to explain these differences (and thus to examine also the more general question of rate behavior in multi-enzyme systems) by identifying the rate-limiting or master reaction in the three tissues. Studies were made of the activity levels and maximum capacities of the individual glycolytic enzymes; comparisons were made (1) of the individual enzymes with each other; (2) of the individual enzymes with the overall glycolytic system; (3) between the time-course of the concentrations of various intermediates and their steady state concentrations as predicted from the kinetics and equilibrium constants of the component enzymes; and (4) of these patterns in normal tissues with those in leukemic tissues.

The results show that hexokinase (HK) is rate-limiting in all three tissues, although its mechanism of rate mastery differs in normal and leukemic cells. In normal cells, HK limits by controlling the rate of glucose phosphorylation. An adequate supply of ADP is maintained by HK (and phosphofructokinase) *and* ATPase activity, which together maintain an optimal ATP:ADP ratio. Conversely, in leukemic cells, the chief consequence of the lowered HK level is a critical lowering of the ADP level since, in these cells, ATPase activity is abnormally low. Thus, ADP formation is doubly impaired and the glycolytic rate is correspondingly diminished.

The data also show that the HMP shunt occurs in leukocytes, though only about 5% of utilized glucose traverses this pathway.

Leukocyte ATPase is being further investigated to determine what relationship, if any, its variations may have to the previously reported variations in leukocyte alkaline phosphatase activity in normal and leukemic cells.

Dynamics of the Hematopoietic System During and Following Leukopheresis in Man

By *H. R. Bierman, K. H. Kelly, B. L. Byron, Jr., F. L. Cordes, D. Schloredt and H. Rattunde.* City of Hope Medical Center, Duarte, California.

The massive and continuous removal of leukocytes (leukopheresis) in dogs has been shown by Craddock et al. to cause stimulation of the bone marrow. Performed in conjunction with chemotherapy, leukopheresis in man by a continuous method has fortuitously permitted a study of the physiologic hemocyto-dynamics in man. Thirteen patients who were hematologically normal were studied on 26 occasions and 6 leukemic patients on 11 occasions. 12 to 1500 billion leukocytes were

removed within 46 to 246 minutes. No serious untoward sequelae were observed.

The behavior of the leukocyte level during leukopheresis was suitable for mathematical analysis in nine nonleukemic patients in 18 instances. This afforded estimates of the size and physiologic characteristics of the leukocyte reservoirs and of the ability of the hematopoietic system to produce and deliver leukocytes into the circulation. It required 126 to 571 minutes for the control leukocyte level to be regained.

In the 6 leukemic subjects, the return of leukocytes to the initial level varied from 100 minutes to 27 days and was, in general, related to the leukemic state. These findings were correlated with changes in the bone marrow, permitting a detailed study of leukopoiesis, erythropoiesis, and thrombocytopoiesis.

Changes in the liver, spleen, and lymph nodes and the significance of the physiologic analysis and correlations with the clinical status of the patients were studied.

Prognosis in the Myelofibrosis-Myeloid Metaplasia Syndrome

By *Robert D. Koler.* Division of Experimental Medicine, University of Oregon Medical School, Portland, Oregon.

Many statements appear in the literature describing a good prognosis for patients with myelofibrosis and myeloid metaplasia, but survival data on a large group of such patients have not been published. From the literature, 119 cases with biopsy diagnosis of myelofibrosis, leukoerythroblastic anemia, splenomegaly, and information sufficient to estimate total survival from onset were collected and analyzed, together with 24 cases we have observed with this disease.

The duration of survival varies from a few months to over 20 years, and the distribution of survival times forms a skewed curve with most of the deaths occurring early and a small number of patients living for long periods. This is essentially the same distribution that has been described for leukemias, lymphomas and other malignant diseases.

By using the maximum likelihood method of Lea, the logarithmic mean, standard error of the mean and standard deviation of our series and of the 119 patients from the literature were calculated and compared with the same parameters calculated by Tivey for 1090 patients with chronic granulocytic leukemia. The 24 patients observed by us have a logarithmic mean of 0.628, a standard deviation of 0.320 and a standard error of 0.0789. The median survival in years with 95% confidence limits is 4.2 (3.02–5.97). The 119 patients from the literature have a logarithmic mean of 0.606, a standard deviation of 0.427 and a standard error of 0.0415. The median survival in years is 4.0 (3.35–4.86). The 1090 patients with chronic granulocytic leukemia were reported by Tivey to have a logarithmic mean of 0.432, a standard deviation of 0.332 and a standard error of 0.0107. The median survival in years was 2.7 (2.58–2.83).

Clinical and Laboratory Studies of Newer Fibrinolytic Agents

By *Burt Cochran, Jr., George D. Ramsay and Arnold G. Ware.* Department of Medicine, College of Medical Evangelists, Los Angeles County General Hospital, and the University of Southern California School of Medicine, Los Angeles.

The fibrinolytic system naturally present in human blood offers a new approach to clotting problems in clinical medicine. The human fibrinolytic system is a potential one, with an inactive plasma precursor, *plasminogen* (profibrinolysin), the active lysin *plasmin* (fibrinolysin), and its plasma inhibitor, *antiplasmin*, all in dynamic equilibrium. This system may be strongly activated by various tissue kinases and bacterial filtrates.

Trypsin and streptokinase-streptodornase have been used to produce fibrinolysis but, because of toxicity and technical problems, are not ideal.

Highly purified protein-free lipopolysaccharides of gram negative bacterial origin can promote fibrinolysis in vivo. Two such pyrogenic substances were used: one derived from E. coli (W-1083) and one from S. abortus equi (W-1064). A detailed pilot study of clinical and laboratory effects was conducted on 12 patients using W-1083. Bleeding and clotting times, platelet counts, prothrombin activity and prothrombin consumption were not significantly affected by intravenous doses ranging from 50 to 300 μg. In only one patient was the fibrinogen lowered at peak fibrinolysis and there was rapid return to normal without mishap.

Subsequently, W-1083 was used repeatedly (102 injections) in 22 patients, most of whom had chronic peripheral vascular diseases. Moderate to complete lysis of fibrin clots was observed (37°C.) in blood drawn 105 minutes after a single injection of 50 to 400 μg. of W-1083 or 0.1 to .5 μg. of W-1064. Side effects, generally mild, consisted of fever, chills, headache, joint pains, malaise, anorexia and somnolence. These studies demonstrate that fibrinolysis can be produced in patients with relative safety.

Current work is aimed at problems of optimal dose and frequency of injections, systemic resistance, and modifying effects of various drugs. Finally, the studies are intended to evaluate the possible role of fibrinolysis in therapy.

The Interference of Unusual Plasma Proteins with the Clotting Mechanism

By *Henry H. Henstell and Miriam Feinstein.* Institute for Medical Research, Cedars of Lebanon

Hospital, and the Department of Medicine, University of Southern California School of Medicine, Los Angeles. (Aided by a grant from Leukemia Research, Inc., Los Angeles.)

The purpose of this work was to study the relationship of unusual plasma globulins to clinical conditions characterized by unexplained hemorrhagic or thrombotic tendencies.

Serum and oxalated plasma samples were used. The unusual globulins were precipitated as euglobulin by water dilution or as cryoglobulin by chilling, or freezing followed by thawing and chilling. Clotting factors were measured by recalcified clotting time, Quick prothrombin test, Ware-Stragnell prothrombin test—which was run as both a one- and two-stage procedure for prothrombin and accelerator activity—and modifications of the Lewis and Ware and Owren methods for factor V and factor VII, respectively. Paper electrophoresis studies were made of all samples.

Nine cases were studied consisting of one case each of macroglobulinemia, multiple myeloma, hyperglobulinemia, uremia and unexplained hemorrhage and four cases of liver cirrhosis.

The precipitable protein was a euglobulin, gamma globulin, in the cases of macroglobulinemia, liver cirrhosis, unexplained hemorrhage and multiple myeloma. The myeloma patient also had a cryoprecipitable fibrinogen-gamma globulin complex. In the cases of uremia and hyperglobulinemia a cryofibrinogen was the major precipitable component.

Precipitation of the euglobulin in five of seven cases and of the cryofibrinogen resulted in a decrease in the prothrombin and accelerator factor activities of the plasma. These clotting factors were then demonstrable in the solutions of the precipitates. Factor V was detected in the precipitates where tested. In one case, precipitation of the euglobulin did not influence the clotting activity of the plasma, although the precipitate contained prothrombin and factor V. In the case of multiple myeloma, euglobulin precipitated from *serum* (100% prothrombin consumption) showed the same order of prothrombin and total accelerator factor activities as did euglobulin from plasma. In the cryoprecipitable complex, prothrombin and accelerator factors were demonstrable while the plasma activity was unchanged.

On the basis of these studies we conclude that (1) unusual globulins have the ability to sequester clotting factors either by in vivo precipitation or in vivo combination; (2) this mechanism could result in hemorrhages due to clotting factor deficiencies and thrombi due to (a) excessive local concentrations of clotting factors or (b) abnormally high concentrations of clotting factors due to combination in the plasma.

Significance of Macroglobulins

By *Arno G. Motulsky, Nils Eriksen, Wade Volwiler and Dennis Donohue.* Departments of Medicine and Pathology, University of Washington, Seattle.

Macroglobulins were occasionally detected by the ultracentrifuge in patients with hematologic malignancy. In order to elucidate their significance a series of previously unreported patients were studied. The clinical course of their disease, bone marrow findings, electrophoretic and ultracentrifuge data were correlated.

When macroglobulins were present in large quantities as demonstrated by a "spike" of high S (>15) value on the ultracentrifugal diagram, the classic clinical picture of macroglobulinemia (Waldenström) was usually present. Patients with classic myelomatosis characteristically had diminished amounts of the normally occurring macroglobulins. Large amounts of an intermediate paraprotein (S 10) were seen in one patient. Mild elevation of macroglobulins was occasionally seen in undifferentiated lymphoma.

A paraprotein band ("peaked" beta or gamma globulin elevation) on serum electrophoresis was found in all typical and atypical macroglobulin patients. These findings indicate the fallacy of diagnosing myeloma from electrophoresis alone. Urinary paraproteins of small molecular size (30,000 to 50,000) were frequently detected; many did not show Bence-Jones solubility.

The bone marrow findings of macroglobulinemia Waldenström were not always pathognomonic; e.g., a myeloma pattern was sometimes seen in macroglobulinemia Waldenström. Conversely, a lymphoid pattern with a few plasma cells supposedly indicative of macroglobulinemia was seen in myelomatosis without macroglobulins. A bone marrow free of abnormal cells was found with lesser amounts of macroglobulins. Pseudoaplastic anemia, as evidenced by pancytopenia and "dry tap" on attempted bone marrow aspiration, was common. Surgical biopsy in such cases showed a marrow filled with abnormal cells. Bone marrow failure was prominent in all macroglobulin patients.

A tall, spikelike macroglobulin (S > 15) on the ultracentrifugal diagram appears to be pathognomonic of the Waldenström macroglobulinemia syndrome. Atypical paraproteins of intermediate size and small elevation of typical macroglobulins may be seen in malignant disease of the reticuloendothelial-plasmacytic apparatus. The elaboration of macroglobulins is not limited to any histologic cell type. Thus, a spectrum of various reticular malignancies may be associated with abnormal protein production. Ultracentrifugal analysis is essential for characterization of such paraproteins.

Occult Blood Loss in Iron Deficiency Anemia of Infancy

By *M. Silvija Hoag, Ralph O. Wallerstein and Myron Pollycove.* San Francisco and Berkeley.

Iron intake, absorption, and maternal factors

have been studied repeatedly in iron deficiency anemia of infancy. The finding of declining hemoglobin mass with hypochromic, microcytic anemia in several otherwise normal infants led to an investigation of the role of blood loss.

All children were aged 6 to 26 months, with hemoglobins ranging from 3.0–9.9 Gm./100 ml. Multiple stool examinations (guaiac method) were performed in the anemic children and a normal control group of comparable ages. All children were placed on meat-free diets starting one day prior to stool collection. Nineteen of the 23 anemic children (83%), and 3 of the 35 nonanemic children (9%), had positive occult blood tests (2–4+). None had melena or frank blood.

Fe^{59} tracer doses (1 μg with specific activity of 2 $\mu c/\mu g$) were given intravenously to 2 of the children. All of the Fe^{59} had been taken up by the red blood cells at one week; 6.09% and 18.6% had appeared in the stools within 4 weeks.

In 3 infants hemoglobin and weights before the onset of anemia were available; total hemoglobins were calculated from the blood volume (estimated as 75 ml./Kg.) and hemoglobin concentration. Total hemoglobin was found to have decreased by more than 40% in all 3 patients (D. C. from 31 Gm. at 8 mos. to 18.4 Gm. at 10 mos.; M. H. from 24 Gm. at 8 mos. to 13 Gm. at 30 mos.; D. D. from 46 Gm. at birth to 26 Gm. at 7 mos.). In 6 other children, birth weights but no previous hemoglobins were available; total hemoglobin at the time of anemia was less than 40% of normal total birth hemoglobin for newborns of comparable birth weights.

These data show active loss of hemoglobin and iron in young children with iron deficiency anemia. While inadequate diet, faulty absorption and decreased prenatal iron stores probably contribute to the development of the anemia, blood loss must be considered a major etiologic factor.

CARDIOVASCULAR SYSTEM

A Simplified Method of Spatial Vectocardiography

By *J. von der Groeben*. Manteca, California. (Aided by a grant from the San Joaquin County Heart Association.)

The great theoretical and didactic value of vectorcardiography on the one hand, and its considerable methodical and technical problems on the other, made us search for a simple procedure that would combine accuracy with practical clinical applicability. By means of a photographic electrocardiograph, recording on electrocardiographic film (Kodak 6 cm.) and a projector, the respective scalar tracings of the 3 spatial coordinates X, Y and Z are projected on a vector drawing instrument, designed for this purpose, which, in a quick semiautomatic manner, plots the frontal, horizontal and sagittal plane projection loops at intervals of 5 milliseconds. Separation of QRS from P and T and clearly discernible time markings make the method in this respect superior to oscilloscopic loops. Plane projections are integrated into a spatial vectordiagram by means of tabulated functions. The whole procedure from the time of recording the leads to the final spatial vectordiagram can be done by a technician, providing the interpreter with an easily readable graph of the spatial loop. Spatial loops, as obtained by this method, were obtained in (1) a series of normals; (2) myocardial infarctions, anterior and posterior wall; (3) hypertrophy, right, left and combined; (4) bundle branch blocks. The advantages of spatial vectordiagrams, obtained in the above described manner, over oscilloscopic plane projection loops are as follows: frontal plane and tilt position and accurate time sequence with clear initial and terminal portion of QRS can be read directly from the spatial vectordiagram, which is a simple graph requiring no perspective or artistic drawing; no oscilloscopes or cameras are required; the recording instrument (2 channel photographic) is portable; the recording can be taken in the office, at the patient's bedside or in the laboratory.

Frontal Plane Vectors from the Low Frequency Ballistocardiograph

By *Don M. Cunningham, Robert Donaldson and Herbert E. Griswold, Jr.* Department of Engineering, University of California, Berkeley, and the Department of Medicine, University of Oregon Medical School, Portland. (Aided by a grant from the U.S.P.H.S.)

As an aid in determining the origin of ballistocardiograph forces, knowledge of the location of the resultant force vector at any time is highly desirable. To this end a three-degree-of-freedom light weight low-frequency suspension was devised for the purpose of determining the line of action of the resultant cardiovascular force in the frontal plane. The body and supporting platform were free to move in the head-foot, medial-lateral and horizontal rotation direction. In each mode of vibration the natural frequency was 0.35–0.36 c.p.s., a frequency well below the ballistocardiograph spectrum but above the normal breathing frequency. Neglecting the minimal restraints on the system, by applying Newton's second law to accelerometer data the resultant force in the frontal plane was found for time intervals of 0.01 seconds from the R wave. The rotational acceleration gave an indication of the moment produced by the resultant force so the quotient moment/force gave

the line of action of the resultant force. If these forces are drawn along their line of action on an outline of the body, the heart and major vessels, much of the earlier conjecture as to their origins is removed. The general movement of blood out of the heart and into aorta and pulmonary artery and the movement of the heart may be synthesized from the position and magnitude of these forces.

Although useful information was obtained from this arrangement, improvement on the accuracy of the results would be desirable. A small percentage of the resultants lie either too far away from any part of the cardiovascular complex to be plausible or else in a region where no force could be predicted at the time of origin based on existing knowledge of blood flow and pressure variations.

Simultaneous Recording of (I^{131}) Transients From Four Crystal Detectors on the Anterior Thoracic Wall

By *Wayne Crockett, Daniel Parrish and Rex L. Huff.* Radioisotope Service, V. A. Hospital, and the Department of Medicine, University of Washington, Seattle.

Three well collimated sodium iodide crystal (1″ x 1″) detectors were placed over areas on the anterior chest wall that were thought to overlie the main body of blood in the right auricle, common pulmonary artery, and the ventricles. A fourth detector was placed away from the major vessels in the region of the second or third interspace about 12 cm. from the midline. Following a single intravenous injection of I^{131} human serum albumin, the signal of each of the four photomultiplier tubes was led through pulse shapers to an Ampex tape recorder running at 60 inches per second. The output of one system was also led to a ratemeter and recorded directly on a single-channel Speedomax inkwriter. Later, all of the Ampex records were played back synchronized with the injection time on the direct record. Playback could be at 60, 30, or 15 inches per second.

Sharp separations between peak activities were evident on some records, e.g., the pulmonary artery and aorta, and ventricles; and the time of occurrence of the peak of the lung transient was between that of the heart chambers. Slow playback of the Ampex record allows demonstration of individual ventricular injections of isotope. Patients with valvular abnormality and specific chamber enlargement have data which appear to be characteristic of the disease. One patient with an interatrial septal defect and anomalous venous return failed to show the sharp separation of peaks. Blood velocities may be calculated on the basis of one-dimensional measurement of distance. Chamber sizes and pulmonary blood volume estimation are being made on the assumption either of linear model and laminar flow or perfect mixing chambers and turbulent flow.

Effects of the Injection of Opaque Media for Selective Angiocardiography into the Dog's Heart

By *Forrest H. Adams, Russell A. McFall, Wallace O. Austin and Bernard J. O'Loughlin.* Departments of Pediatrics and Radiology, U.C.L.A. School of Medicine, Los Angeles. (Aided by grant from the Los Angeles County Heart Association.)

The study to be reported was undertaken in an effort to determine, under controlled conditions, the effect of injecting radiopaque material under high pressure into the dog's heart. Answers to certain questions seemed important because of the recent increased use of the technic of selective angiocardiography in the diagnosis of certain forms of heart disease.

Radiopaque material was injected into the various chambers and great vessels on both the right and left sides of the dog's heart while, at the same time, pressures in two of the other chambers were recorded. 38 dogs were used in 68 experiments.

Cardiac irregularities with a concomitant alteration in right ventricular and systemic pressure were encountered in a high percentage of the injections into the superior vena cava, right atrium, right ventricle, pulmonary artery, and left ventricle. When the injections were made into the inferior vena cava, or aorta, these alterations were not observed. Subendocardial hemorrhages were frequently observed in those dogs' hearts in which the catheters were allowed to remain for longer than one hour.

Although selective angiocardiography has definite advantages over earlier technics, these studies in dogs would suggest certain limitations if the injections are made into certain sites.

Transthoracic Catheterization

By *Hans H. Hecht and Ramon L. Lange.* Department of Medicine, University of Utah College of Medicine, Salt Lake City.

Left and right atrial and ventricular catheterization have been carried out percutaneously in 55 patients, using as landmarks the right paravertebral line, an angle of 15°, and the cardiac center (Bjoerk, Fisher). The procedure is combined with right-sided cardiac catheterization and central pulse recording. An optimally damped strain gage catheter system of equal sensitivity is used for all three procedures. In order to estimate retrograde flux through an incompetent mitral valve, a double atrial puncture is performed whereby a catheter placed in the ventricle serves to inject the indicator substance, and dye contamination of atrial blood is determined from an indwelling atrial needle and a direct recording oximeter. The area of reflux may be expressed as a percentage of the area of forward flow which, in turn, is determined by dye injection into the right ventricle and sampling from the left atrium. In a low pressure

venous system differences in form and magnitude between pulmonary wedge pressures and left atrial pressures may be observed, but identical values are obtained when pressures are high, either because mitral stenosis is present or tension is raised by norepinephrine. Occasionally, a regurgitant jet of mitral insufficiency may be seen, and a characteristic valve artefact resembling the pattern of infundibular stenosis may be recognized. The tandard orifice formula $Q = KA\sqrt{2gP_1 - P_2}$ clearly indicates that any valve gradient depends on flow across the orifice and that pressure data cannot be interpreted without flow measurements. On the other hand, the data allow calculation of flow during atrial contraction and permit the assessment of the contribution of atrial systole to forward flow.

The procedure, although harmless, is uncomfortable for all concerned. There was no fatality and no pneumothorax occurred. Hemothorax was noted once, and intercostal neuralgia of a few hours duration was frequent.

Observations on the Hemodynamics of Corrected Transposition of the Great Vessels Associated with Intracardiac Defects

By *Burton W. Fink, Forrest H. Adams, Russell A. McFall and Bernard J. O'Loughlin.* Departments of Pediatrics and Radiology, U.C.L.A. School of Medicine, Los Angeles.

The purpose of this report is to describe a poorly understood congenital heart malformation referred to as "corrected transposition of the great vessels" and to show how it can be differentiated from simple interventricular septal defect with a left to right shunt, and from true transposition of the great vessels. Five patients were studied by cardiac catheterization and selective angiocardiography; 2 of these were explored surgically. One patient expired and has been studied at post mortem examination.

In all of the patients the course of the cardiac catheter was medial and somewhat posterior as it entered the pulmonary artery from the right ventricle. Likewise, all had a significant left to right interventricular shunt with right ventricular and pulmonary hypertension. Selective angiocardiography demonstrated the anatomical position of the pulmonary artery and the aorta in each case.

Autopsy examination in the one patient demonstrated the transposed nature of the atrioventricular valves and of the ventricular myocardium and endocardium on the two sides.

Proper clinical recognition of corrected transposition of the great vessels associated with an interventricular septal defect is essential, now that corrective surgery is possible for simple interventricular septal defect. It is possible to make such a diagnosis by the combined use of cardiac catheterization and selective angiocardiography.

Stenosis of a Branch of the Pulmonary Artery

By *Frederic L. Eldridge, Arthur Selzer and Herbert N. Hultgren.* Department of Medicine, Stanford University School of Medicine, San Francisco.

Reports of stenosis of a branch of the pulmonary artery have been rare and it has been assumed that the condition is very uncommon. Nevertheless, during the past year, cardiac catheterization studies in this laboratory have revealed five children who exhibited sharply localized pressure gradients in one or both branches of the pulmonary artery, indicating the presence of one or more stenoses of these branches.

All of the patients had other congenital cardiac anomalies and many of the signs and laboratory findings were related to these lesions. All had systolic ejection murmurs which could have been caused by either the cardiac anomaly or the pulmonary artery branch stenosis, but two patients also exhibited continuous ductuslike murmurs at the chest wall over the site of the stenosis. One of these patients had been previously explored for patent ductus arteriosus, which had not been found.

The characteristic hemodynamic feature of this lesion consisted of a pressure rise as the catheter was withdrawn from a distal position to a more proximal portion of a pulmonary artery branch, or a pressure rise as the catheter was moved from a branch to the main pulmonary artery. In all cases, the pressure change always occurred at the same spot and was of the same magnitude on repeated withdrawal tracings. All cases exhibited systolic pressure gradients across the area of stenosis, but two had diastolic pressure gradients as well. These two patients were the ones possessing continuous murmurs. Evidence of stenosis was found in the left pulmonary artery in one case, in the right in three cases, and bilaterally in another case. One patient had evidence of two areas of stenosis on the same side. Pulmonary hypertension ranging from mild to moderate in degree was found in three of the five patients.

On the basis of data obtained from the five patients described above, as well as a review of 10 other patients obtained from the literature and by personal communication (including one autopsy study), the following tentative conclusions can be made:

(1) Segmental stenosis of a branch of the pulmonary artery is a new clinical entity. (2) It is probably a congenital lesion; it occurs frequently in conjunction with other congenital cardiac defects; it may produce characteristic murmurs, alterations of pressure and an increase in RV work. (3) The diagnosis can be made most easily by cardiac catheterization and selective angiocardiog-

raphy. (4) Autopsy data of such lesions is rare because serious cardiac impairment and death rarely occur and because previously such lesions have probably been overlooked.

Masked Mitral Stenosis: The Pulmonary Hypertensive Heart and Mitral Insufficiency

By *Ramon L. Lange, R. Lawrence Sifford and Hans H. Hecht.* Department of Medicine, University of Utah College of Medicine, Salt Lake City.

The proven worth of mitral commissurotomy in mitral valvular obstruction, despite the severity of the disease on one hand, and the often unexpected post-mortem finding of severe mitral stenosis on the other, allows wide latitude in the search for this potentially "curable" lesion. Because of the rapid downhill course exhibited by a group of patients with long-standing heart-failure, severe cardiomegaly, loud apical systolic murmur, and clinical evidence of severe pulmonary hypertension, right and left heart catheterization was performed in ten cases in order that the relative importance of specific valvular lesions could be assayed. After routine hemodynamic data were obtained via right heart catheterization, the left atrium and ventricle were entered by paraspinal approach and mitral valve diastolic pressure gradients were measured. Subsequently, the amount of regurgitated left ventricular stroke volume was estimated from the dye curve obtained by left ventricular injection and left atrial sampling, as compared with the total "forward" dye curve.

Two patterns emerged from this group of patients who for years had been considered to have either predominant or "pure" mitral incompetence. (1) Tight mitral stenosis with very high valve gradient, no valve incompetence, severe pulmonary hypertension, cardiomegaly, high pitched apical systolic murmur, absent or elusive apical diastolic murmur and severe, long standing congestive failure. (2) Moderate mitral stenosis with high valve gradient and moderate degree of mitral insufficiency (less than 25% of stroke volume), with the other characteristics of Group (1). In these cases the increased diastolic flow into the left ventricle (caused by regurgitation during systole) results in a gradient considerably higher than would be expected from the valve area and net forward flow values. In these cases, wide opening of the valve at surgery is essential for improvement.

These elaborate investigative diagnostic procedures have resulted in the discovery of lesions which are amenable to present-day surgical procedures. These patients are representative of an otherwise discouraging clinical group who hitherto displayed confusing diagnostic signs but who may now be grouped together as a clinically recognizable syndrome that responds well to surgery.

Measurement of Back-Flow in the Aorta Associated with Insufficiency of the Aortic Valve

By *Homer R. Warner and Alan F. Toronto.* Department of Physiology, Latter Day Saints Hospital and University of Utah, Salt Lake City.

Aortic valvular insufficiency is characterized by flow of blood during diastole from the root of the aorta back into the left ventricle. As the insufficiency becomes greater, back-flow should occur in progressively more distal segments of the aorta. On this premise a technic has been devised for determining the magnitude of back-flow in segments of the descending thoracic aorta.

Evans' blue dye injections are made through an arterial catheter which has been advanced through a needle in the right femoral artery into the aorta approximately to the origin of the left subclavian artery. Indwelling needles in the left radial (human) or left carotid (dogs) and left femoral arteries are attached to cuvette oximeters for continuous recording of dye concentration at each of these sites following injection into the aorta. The injection device delivers 0.622 cc. (6.22 mg. dye) in 0.1 sec. The onset of injection is triggered by the R wave of the E.C.G. with a delay of 0.1, 0.4, or 0.6 second.

The ratio (R) of the area under the time concentration curve at the radial or carotid artery to that at the femoral artery is an index to the fraction of injected indicator going to each recording site. Injections are made at various points as the catheter is withdrawn measured distances (X) down the aorta. It has been found that the logarithm of R decreased linearly with X. This slope in normal subjects was greater than 1.0 (i.e., 100% decrease in ratio per cm. withdrawn). In patients with severe aortic insufficiency and in dogs with one leaflet of the aortic valve incised to the ring, the slope was less than 0.2.

Calcific Valvular Disease in the Patient Over 80

By *Herbert N. Hultgren.* Department of Medicine, Stanford University School of Medicine, San Francisco.

Despite the fact that calcific valvular disease is one of the most common cardiac disorders affecting our rapidly aging population, little information is available concerning the incidence, distribution, size, and characteristics of calcific deposits in the heart valves of the very elderly. For this reason a careful anatomic study has been made of hearts from an unselected autopsy series of 50 patients over 80 (mean age 83.5 years), consisting of 30 males and 20 females. This age group was selected because of its high incidence of valvular calcification. Aortic and mitral valves and valve rings were dissected and grossly visible calcific deposits were recorded. Clinical and pathologic

correlations were examined. The following observations were made:

Calcification of the mitral valve and annulus was more common in women (50%) than in men (24%), and massive calcification of the annulus was much more common in women (35%) than in men (3.5%). Aortic valvular calcification was as frequent in women (50%) as in men (48%), and massive calcification of the aortic valve resulting in obstruction to blood flow also showed no sex difference (women 5% and men 7%). All gradations of calcific aortic disease from small pocket calcifications to severe calcific aortic stenosis were noted. A relation to rheumatic valvular disease could not be established. Calcific deposits in either aortic or mitral valves greatly increased the likelihood of encountering calcific deposits in the other valve, thus suggesting a common etiology.

These observations support previous work demonstrating the high incidence of valvular calcification in old age and the unusual frequency and predilection for calcification of the mitral annulus in the elderly female. Aortic stenosis in the aged subject is probably an advanced stage of an atherosclerotic calcific lesion that increases in incidence with advancing age and that is associated with calcific deposits in the mitral valve and annulus; both these processes probably have a common nonrheumatic etiologic background.

Paroxysmal Rapid Ventricular Response in Patients with Atrial Fibrillation as a Paradoxical Manifestation of Digitalis Intoxication. Report of Four Cases Successfully Treated with Intravenous Potassium

By *Marvin B. Bacaner*. Medical Service of the Mount Sinai Hospital, New York City, and Stanford University Hospitals, San Francisco.

The usual action of the cardiac glycosides in patients with atrial fibrillation and heart failure is to increase the block at the atrio-ventricular node, which slows the ventricular response. However, four patients with long standing atrial fibrillation and advanced congestive heart failure were observed with a sudden marked increase in ventricular response, despite having received large doses of cardiac glycosides. In each case the paroxysm occurred 6 to 24 hours following mercurial diuresis.

Since there was no indication that additional digitalis would slow the ventricular rate in these patients, the possibility that the increase in ventricular response was a manifestation of digitalis intoxication was considered. In order to investigate this possibility, digitalis and mercurials were discontinued and an intravenous infusion of 40 mEq. of potassium in 500 cc. of 5% dextrose in water was administered to each patient.

Potassium administration progressively decreased the ventricular response within two hours in each case. Case I had a ventricular response of 200 prior to treatment which decreased to 80. Case II had a ventricular rate of 180 which decreased to 85. Case III had a ventricular response of 160 which slowed to 90. Case IV had a ventricular rate of 160 which decreased to 85. In one case, after initially slowing the ventricular response with potassium, administration of intravenous digoxin caused the rate to increase to the pretreatment level. This rapid ventricular rate was again slowed by additional potassium.

The evidence indicates that paroxysmal or progressive increase in ventricular response in patients with atrial fibrillation may be a manifestation of digitalis toxicity. This generally unrecognized toxic potential of the cardiac glycosides represents a potentially dangerous paradox. Since it simulates a situation in which the tendency would be to intensify digitalis administration, it may lead to fatal overdosage. The association in onset of the paroxysmal event with recent mercurial diuresis suggests that potassium losses in the diuretic urine may have potentiated the effect of the digitalis already administered and may explain the salutary effect of potassium therapy.

Evaluation of Cardiac Reserve

By *Robert A. Bruce, Theodore J. Fuller, William W. Andrus, Curt A. Wiederhielm and Charlotte Hamilton*. Division of Cardiology, University of Washington, Seattle.

Cardiac reserve is defined as the capacity to increase both stroke index and heart rate and to maintain a high venous oxygen reserve in response to increased metabolic demands. The degree of impaired reserve was studied in 13 patients with either rheumatic or coronary heart disease by comparing these parameters during a steady state of exercise while walking for several minutes on a treadmill at 1.7 mph and 10% grade with the corresponding measurements obtained while sitting in a chair. Ventilation and oxygen consumption were determined by a respirometer. Cardiac outputs were derived from T-1824 time concentration curves obtained with an ear oximeter, Wiederhielm's amplifier with linear characteristics and a 10-inch strip recorder. Arteriovenous oxygen differences were estimated by Fick principle. Since both hemoglobin concentration and arterial oxygen saturation were measured, venous oxygen reserves were calculated by subtracting AV differences from arterial oxygen contents.

Cardiac index at rest averaged 2.9 (range 2.0 to 4.0) L/M²/minute in cardiacs, versus 3.6 (2.6 to 5.2) in noncardiac subjects. It was not possible to predict exercise tolerance or cardiac reserve from resting measurements in these cases. Cardiacs with Class III functional capacity and impaired exercise tolerance (physical fitness index [PFI] 3 to 5) exhibited a 23% fall in stroke index, despite a slight rise in cardiac index. Class II cardiacs with normal exercise tolerance (PFI 23 to 26) exhibited 37% increase in stroke index, considerable increase

in cardiac index, with variable changes in heart rate and venous oxygen reserve. Others with intermediate exercise tolerance (PFI 12 to 19) in Classes I to III, by subjective criteria, exhibited intermediate changes with predominant adjustments varying with increase in stroke index or reduction in venous oxygen reserve. Since similar variations in circulatory adjustments to exercise have been observed in noncardiac patients, the importance of other factors and the need for quantitative measurements are apparent.

It is concluded that a method of quantitatively appraising reserve capacity of ambulatory cardiac patients is now available.

Hemodynamic Patterns in Clinically Controlled Left Ventricular Failure

By *Arthur Selzer and Donald J. McCaughey*. Medical Service, V. A. Hospital, and the Department of Medicine, Stanford University School of Medicine, San Francisco. (Aided by a grant from the San Mateo County Heart Association.)

This study was undertaken in order to investigate a possible relationship between circulatory dynamics and clinical features of cardiac failure. For the sake of uniformity, cases were limited to primarily leftsided cardiac lesions, such as failure due to hypertensive and coronary diseases and valve defects of the left heart exclusive of mitral stenosis. Furthermore, patients were investigated when the clinical condition and, presumably, the hemodynamic status reached a stable stage, that is, when the maximum benefit from cardiac therapy was obtained, and/or the patients' dry weight maintained for some time.

Circulatory dynamics in the 57 patients thus investigated fell into three main categories: (1) Normal output group, which included patients with a normal resting cardiac index: (a) with normal circulatory dynamics on exercise (6 cases); (b) with an abnormal response to exercise (4 cases) and (c) with abnormally elevated pressures at rest, during exercise, or both (10 cases). (2) Low output group, which included 13 patients with normal pressures and 15 patients with elevated pressures in the pulmonary arterial or venous systems but with normal gradients and resistances. (3) Pulmonary hypertensive group, which included 9 patients with significantly elevated pulmonary vascular resistance.

It is seen that in addition to the expected quantitative changes in circulatory dynamics, important qualitative alterations occur in cardiac failure. As in mitral stenosis, elevation of left atrial pressure occurring in left ventricular failure appears to lead to an elevation of pulmonary vascular resistance in some cases but not in others. In this series the pulmonary hypertensive group, which comprised ⅙ of the patients, did not appear to be related to the etiology of cardiac failure, the duration of symptomatology or the severity of failure as judged by clinical criteria or by physiologic measurements.

Production of Congestive Heart Failure in the Dog by Ultrasound

By *H. Lenox Dick, James D. Krueger and Elton L. McCawley*. Department of Pharmacology, University of Oregon Medical School, Portland.

Congestive heart failure has been produced in the dog by irradiation with 1 megacycle ultrasound applied to the exposed pericardium. The congestive failure that develops apparently has all of the characteristics of clinical failure and responds in the same manner to digitalis.

Ultrasound, 3 watts/cm.^{2}, 12.5 cm. in area for 26 minutes, was applied to the left ventricle. Angular rotation of the sound head through saline coupling provided a greater effect on the proximal epicardial surface than on the other portions of the heart. Air bubble decoupling was necessary to prevent rib-intercostal muscle separation and "exit" ulcers. Under these conditions there are occasional arrhythmias and one dog died from ventricular fibrillation. Grossly, the lesion resembles a diffuse myocardial infarct; microscopically, there is coagulation necrosis with no suggestion of vascular occlusion and subsequent infarction.

Signs of congestive failure appear after two to four weeks. The severity of the failure depends on the extent of the lesion (30–50%); some dogs remain compensated with the addition of sodium chloride imposed an additional load on the heart.

Treated dogs developed the following signs and symptoms: 10–16% increase in body weight with hepatic enlargement; cardiomegaly of nonirradiated areas of the heart; exertional dyspnea and cyanosis; a functional apical murmur; and elevated venous pressure.

The administration of acetylstrophanthidin was followed by a decrease in venous pressure, an increase in systolic pressure with a smaller rise in diastolic pressure, and greater stroke volume output. Digitalization with digoxin was followed by a 10–15% weight loss (diuresis), increased exercise tolerance, disappearance of murmur, and bradycardia. Overdigitalization provoked nodal rhythms, extra systoles or ventricular tachycardia, which could be abolished by potassium chloride administration.

Simultaneous Human SGO-T, SGP-T and SLDH in Doubtful or Complicated Myocardial Infarction

By *J. J. Sampson, H. Weisberg, A. Lieberthal and A. E. Lewis*. Departments of Medicine and

Pathology and the Harold Brunn Cardiovascular Institute, Mt. Zion Hospital, San Francisco.

Damage of various degrees of different tissues results in different maximum peaks and patterns of concentration of serum glutamic oxalacetic transaminase (SGO-T), serum lactic dehydrogenase (SLDH), and serum glutamic pyruvic transaminase (SGP-T), especially of the latter. These different concentrations indicate the diagnosis of myocardial infarction, either small and atypical or complicated by other disease, especially of the liver.

The technics employed in determinations of these serum enzymes were those reported by LaDue and associates, except that the pyruvic reaction was reversed. Spectrophotometric measurement was employed as an indicator of the timed decrease of the density of reduced diphosphonucleotide (DPNH) during its oxygenation in the final reactions. Serial determinations at 4–8 hour intervals for one to three days after the presumptive onset of an attack are essential. Serum or blood for LDH testing must be refrigerated within one hour.

Only SGO-T determinations were made in 64 patients, including 16 normal adults. Thirty-four studies were made on 32 patients for SGO-T, SLDH, and SGP-T simultaneously. Included were two normal subjects and 22 with suggestive or confirmed myocardial infarctions in whom immediate (12–24 hours) and characteristic short curves of SGO-T and SLDH generally varied in height and duration with the severity of the attack. Approximately one half of the patients with suggestive histories but doubtful "T-wave" electrocardiograms presented diagnostic curves. Elevation of SLDH persisted one to three days longer than SGO-T.

SGP-T is elevated only slightly in myocardial infarctions, unless complicated by shock and failure or chronic hepatic disease, when higher but late or irregular curves are noted. SGP-T is invariably high in maximal concentration in acute hepatitis. SLDH at times exhibited independent moderately high curves in unrelated acute febrile illnesses.

Conclusion: the different specific patterns of concentration curves of SGO-T, SLDH, and SGP-T serve as valuable diagnostic aids in cases of doubtful or complicated myocardial infarction and may be useful in differentiating hepatic and other diseases.

Endocrine and Lipid Aspects of Experimental Atherosclerosis in Dogs

By *Sheldon Rosenfeld, Jessie Marmorston, Harry Sobel, John Mehl, Jack Lewis and Albert White.* Institute for Medical Research, Cedars of Lebanon Hospital, and the University of Southern California, Los Angeles.

This presentation attempts to answer the following questions arising in the course of a study of the endocrine and lipid aspects of experimental atherosclerosis in dogs: (1) Is thiouracil as effective as thyroidectomy in producing hypercholesterolemia in the cholesterol-fed dog? (2) Is there a sex difference in susceptibility to the development of experimental hypercholesterolemia? (3) What is the role of the adrenal and, particularly, ACTH in experimental hypercholesterolemia?

Fifty-eight dogs (33 beagles and 25 mongrels) were divided into the following groups and studied for from six to twelve months: normal (12 months); thiouracil feeding (10 months); thyroidectomy (6 months); thiouracil plus cholesterol feeding (10 months); thyroidectomy plus cholesterol feeding (6 months); ACTH, 40 units 1–3 doses, followed in 2–4 months by thiouracil and cholesterol feeding (6 months). Serum cholesterol, phospholipid and alpha and beta lipoprotein determinations were made at monthly intervals. At three-month intervals, 24-hour urine collections were made for estrogen and corticoid determinations.

The average serum cholesterol levels observed during the course of the experiments were as follows: in the normal dog, 150–200 mg.%. In thiouracil-fed, or thyroidectomized dogs, 300–400 mg.%. Thiouracil plus cholesterol feeding induced a rise to 500–700 mg.%. Thyroidectomy plus cholesterol feeding produced a rise to 900 mg.% in male dogs and 550 mg.% in female dogs. In dogs pretreated with ACTH and then fed thiouracil and cholesterol, the levels rose to an average of 1400 mg.%.

It is concluded that thiouracil feeding is less effective than thyroidectomy in producing hypercholesterolemia in cholesterol-fed dogs; that thyroidectomized dogs fed a high cholesterol diet appear to show a male-female difference in susceptibility to the development of hypercholesterolemia; and that pretreatment with ACTH greatly enhances the hypercholesterolemia in thiouracil plus cholesterol-fed dogs.

A Study of the Valsalva Maneuver in Hypertension

By *Stephen R. Elek and Richard N. Baum.* Cardiac Clinic, Cedars of Lebanon Hospital, Los Angeles.

The purpose of this paper is to study the effect of orally administering anti-hypertensive agents on the poststraining phase of the Valsalva maneuver in hypertensive patients. Eleven patients from 32–76 years of age with essential and renal hypertension were examined. The drug used was hexamethonium (125 mg.) combined with Reserpine (0.125 mg.) in one tablet. Dosage range of hexamethonium was 375–750 mg. and of Reserpine 0.375 mg. given daily for two-week periods alternating with fortnightly control periods using placebo. In all patients, the response to the Valsalva maneuver was tested before administering the drugs or placebo.

All patients in whom the poststraining bradycardia could be inhibited by the antihypertensive

drug showed an excellent or satisfactory reduction of blood pressure except one; the latter had no hypotensive effect and it is likely that the dosage of the specific medication was insufficient. The three patients who exhibited the most marked control poststraining bradycardia had the best response to the antihypertensive drug. Four patients did not have the usual poststraining bradycardia in the control interval; three of these individuals had a poor response to the drug and one patient had only a fair response. In three patients given reserpine alone, the poststraining bradycardia was also inhibited but the reduction in blood pressure was only fair.

The data suggest that the simple Valsalva maneuver is a useful method of anticipating which hypertensive patients will respond to blood pressure lowering drugs. This study has interesting implications with respect to the neurogenic pathways in hypertension.

Control of Conditioned Responses of Digital Blood Vessels

By *Travis Winsor*, Los Angeles.

Studies were made to determine whether conditioned vasoconstrictive responses to a conditioned stimulus could be blocked without blocking vasoconstrictive responses to the unconditioned stimulus. These studies were carried out while searching for a means of controlling certain disorders, such as Raynaud's Disease, which may represent abnormalities of the conditioning mechanism.

A digit was sealed in a plethysmographic cup and volume changes were recorded continuously. Conditioned responses were produced by administering simultaneously an unconditioned stimulus (electric shock) with a conditioned stimulus (buzzer). The conditioned stimulus produced no constriction unless both stimuli had been previously administered simultaneously. Certain drugs were employed to prevent the development of conditioned responses or to block the conditioned responses after they had been formed. Benzilic acid diethylaminoethylester hydrochloride (Benactyzine) and alcohol blocked the development of the conditioned response. The phenothiazines, 10-(3-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride (Thorazine), 2-chloro-10-3′-(n-methylpiperazinyl)-propyl-phenothiazine dimaleate (Compazine), methoxyphenothiazine, n-methylpiperidyl-3-methylphenothiazine (Pacatal) and dextroamphetamine sulfate (Dexedrine) and 2-amino-1-(3,4-methylene-dioxyphenyl)-propane hydrochloride (SKF-5) blocked the conditioned response after it had been formed without interfering with the response to the unconditioned stimulus. Nembutal and placebos had no effect on the development or inhibition of the conditioned responses.

It is concluded that digital vasoconstrictive conditioned responses can be controlled by certain agents which have a central site of action. These agents may be useful in those disease states in which abnormalities of the conditioning mechanism exist or where vasoconstrictive responses are undesirable.

ENDOCRINES AND METABOLISM

The Concentration and Binding of Thyroxine and Triiodothyronine by Rat Diaphragm

By *John R. Hogness, Norman D. Lee, Margaret Berg and Robert H. Williams.* Department of Medicine, University of Washington School of Medicine, Seattle.

Since one of the most important sites of thyroid hormone action is in the muscle mass of the body, it seemed of interest to study the nature of the binding phenomenon exhibited by rat diaphragm in vitro relative to thyroxine and triiodothyronine labeled with I^{131}.

Studies of the uptake of these two compounds by rat diaphragm as a function of incubation time, hormone concentration in the medium and duration of rinsing, as well as equilibrium studies, were carried out. The incubation studies showed that at all concentrations of thyroxine studied the amount of the hormone bound was linearly related to time of incubation. Concentration studies also indicated a direct linear relationship between the amount of thyroxine available and the amount bound to tissue. Washout studies showed that, with prolonged rinsing, little thyroxine was removed from rat muscle after the first 15 minutes. Equilibrium studies, wherein hemidiaphragms were incubated first with labeled thyroxine and then transferred to solutions containing unlabeled material, showed no significant loss of initial radioactivity during incubation with the nonradioactive thyroxine. All of these observations indicated that thyroxine is indeed specifically bound to rat muscle tissue in concentrations far exceeding those in the incubating medium.

Similar incubation and washout studies were carried out with triiodothyronine. These studies showed not only that triiodothyronine was also specifically bound to rat muscle tissue but that this binding took place much more rapidly than in the case of thyroxine. This finding is in accord with previous observations regarding the relative rates of action of these two compounds in vivo.

Comparison studies carried out with Na I^{131} showed that, in equimolar concentrations, relatively insignificant amounts of this compound were taken up by rat diaphragm and that which was adsorbed was readily removed by washing.

Hyperthroidism in the Aged

By *Loren T. DeWind, Robert R. Commons and Paul Starr.* University of Southern California School of Medicine, Los Angeles.

An analysis is presented of the case histories of 49 patients aged 61 and over who were treated by the Thyroid Group at the Los Angeles County General Hospital between the years 1948 and 1956. There were 44 women and five men in the group.

The symptoms were typical of hyperthyroidism in slightly more than half, but in one-third were chiefly cardiovascular, and in one-tenth suggested nervous or mental disorder. The most prominent symptoms were weight loss, dyspnea, instability and weakness. Of 45 patients with goiter, it was described as diffuse in 17 (38%) and nodular in 26 (62%). Complicating diseases were varied, with diabetes occurring in six (12%).

Blood protein-bound iodine ranged between 5.1 and 30 μg. %, with most between 9.1 and 14.0. When the protein-bound iodine was less than 8.0 the diagnosis was equivocal or the patient was taking mercurials or antithyroid drugs. Twenty-four hour uptakes of I^{131} varied from 21% to 90% with most between 41 and 70%.

Most patients achieved euthyroidism within six months. Retreatment was necessary in nine. Six became hypothyroid. There were three deaths known to us. One who was considered critically ill when treated died six days after treatment. One died three months after treatment in uncontrolled congestive failure. One died four months after treatment from a cerebral vascular accident.

Conclusions: Hyperthyroidism causes significant morbidity in the aging population; the symptoms are atypical in a significant percentage of patients; the presence of complicating diseases makes diagnosis more difficult; the blood protein-bound iodine is the most accurate indication of hyperthyroidism in the absence of mercurial or antithyroid drugs; and radioactive iodine is a safe and effective form of therapy.

The Effects of the Sulfonylureas on Thyroid Function in Man

By *Josiah Brown and David H. Solomon.* Department of Medicine, University of California Medical Center, Los Angeles.

The purpose of this study was to measure the effects of carbutamide (BZ55) and tolbutamide (Orinase) on thyroid function in human subjects, most of whom were under treatment for diabetes mellitus.

The uptake of radioiodine at 2, 4, 6, 8, and 24 hours was measured before sulfonylurea therapy, during the acute administration of propylthiouracil and at 7- to 40-day intervals during therapy. Serum protein-bound iodine measurements were made at similar intervals. Thirteen patients, most of whom were given both drugs in several doses, have been studied for 4–7 months.

Of 13 patients who received tolbutamide, 5 had a significant block in uptake of I^{131}. Two had a fall in serum P.B.I. of over 1 μg.% and in 2 additional cases the P.B.I. was held down after having originally fallen on carbutamide. Of 11 patients on carbutamide, 7 were blocked and 2 more had a marked rebound above control when the drug was stopped. Five out of 10 patients had a depression of serum P.B.I. on this drug. There were no effects on thyroid function from the daily administration of 1 Gm. of tolbutamide, but 2 Gm. of tolbutamide or 1 Gm. of carbutamide were antithyroid in some patients. In summary, both compounds have anti-thyroid activity in human subjects with carbutamide being approximately 2 times as potent as tolbutamide.

The effect of 3 Gm. of tolbutamide and carbutamide on the "accumulation gradient" of I^{131} was determined at the start of therapy in 9 subjects. The acute effect of both drugs was slight, whereas after 3–7 days the antithyroid effects were definite. Acute studies of the mechanism of action of carbutamide in 2 hyperthyroid patients revealed neither a thiocyanatelike nor a thiouracillike effect on thyroid function.

Parathyroid Function in Malabsorption Osteomalacia

By *Joseph Picchi, Jackson Crane, G. S. Gordan, H. Q. Sakai and Howard Steinbach.* Endocrine Clinic of the Department of Medicine, and the Departments of Pathology and Radiology, University of California School of Medicine, San Francisco.

Malabsorption osteomalacia following peptic ulcer operations in 3 cases and associated with steatorrhea in another case was characterized by bone pain, multiple fractures following minimal trauma, high alkaline phosphatase levels, and a low serum (calcium × phosphorus) product. Calcium infusion showed avidity for calcium and a subnormal serum phosphate rise. Iliac bone biopsies demonstrated softening (malacia) and radiolucency, but decalcified sections failed to show osteoid seams. Tubular reabsorption of phosphate (TRP), which rises virtually to unity in the absence of parathyroid function and which is decreased in hyperparathyroidism, reflected the rise normally

seen in phosphate deprivation in 2 cases. Two other patients had a low TRP and normal serum phosphorus levels.

It appears that phosphate malabsorption initially depresses parathyroid function and thus conserves phosphate, just as in phosphate deprivation in normal individuals. Later, a critical hypocalcemia stimulates parathyroid activity with a resultant phosphate diuresis at the expense of bone. Viewed thus, phosphate diuresis is a secondary event in osteomalacia caused by primary intestinal malabsorption rather than the cause of osteomalacia, as proposed by Dent. This concept also explains the paradoxical rise of TRP following administration of vitamin D in osteomalacia, as observed by Fraser and Nordin.

A Study of Insulin Metabolism Using Insulin-I^{131}

By *Arne N. Wick and Douglas R. Drury.* Scripps Clinic and Research Foundation, La Jolla, California, and the Department of Physiology, University of Southern California, Los Angeles.

Radioactive iodinated insulin has been extensively used to study the dynamics and metabolism of natural insulin in tissues. In the intact animal its use is somewhat limited because it is rapidly degraded. In the eviscerated-nephrectomized rabbit the degradation rate is relatively slow so that it is possible to measure the rate of mixing and the volume of distribution with greater accuracy. Iodinated insulin leaves the blood stream at about the same rate as inulin so that its concentration in the plasma levels off about 1 hour after injection. The concentration at this time indicates dilution by a volume equivalent to 20% of the body weight. This means that no appreciable amount of the iodo-insulin can be intracellular or bound on cell surfaces at this time when natural insulin shows maximal biological activity. In control animals injected sodium $iodide^{131}$ distributes in a volume equivalent to 25% to 30% of the body weight in 15 minutes and increases only slightly with time. The biological decay curve of iodinated insulin (TCA precipitable) parallels that for biological activity of natural insulin after small doses in normal and in eviscerated rabbits. This suggests that iodo-insulin is degraded at the same rate as natural insulin. Natural insulin in small doses reaches peak biological activity within a few minutes after its injection, at which time iodo-insulin has an apparent volume of distribution equivalent to little more than the plasma volume. If a stable insulin-tissue binding occurred, the biological activity of small doses of insulin should increase with time. It is difficult to reconcile our observation with the concept of a stable binding of insulin by tissues playing any significant role in insulin action.

The Human Bio-Assay of ACTH

By *Ernest M. Gold, Paul Starr, Arnold G. Ware and S. R. Notrica.* University of Southern California School of Medicine, Los Angeles.

This study has developed a bio-assay method that is objective, simple, reproducible and relatively inexpensive. A group of normal young adult male and female subjects were selected. Normality was verified by medical history, examination and certain laboratory procedures. The mean control urinary excretion of adrenocortical steroids was determined for each subject from specimens collected for two consecutive 12-hour periods on at least two separate days. Urine was assayed for both 17-ketosteroids and 17-ketogenic steroids according to the method of Norymberski.

A curve of graded responses to increasing doses of ACTH was then established for each individual. The preparation used as a standard was arbitrarily selected from a single lot produced by a large manufacturer for commercial sale. It was found that the urinary steroid excretion during the first 12 hours following the injection of ACTH was most sensitive as an index of response. Additionally, it was found that the 17-ketosteroid response to ACTH was sluggish and unpredictable. For these reasons, the dose-response curves were based upon the 17-ketogenic steroid excretion in the immediate daytime 12-hour period after ACTH administration.

Samples from unknown or new ACTH lots were then assayed for potency, based on comparison of 17-ketogenic steroid excretion with graded doses of the reference batch. The assayed potency of the unknown sample was then interpolated from the curve already determined for the subject being used. Based upon total mg. of 17-ketogenic steroid output as compared with the reference batch, an unknown may be reported in terms of (1) *H.S.P. units per cc.* (Human Steroidogenic Potency), primarily a modification of U.S.P. units; (2) *Per Cent of Labeled Potency*—the ratio between assayed H.S.P. units and the U.S.P. unit potency displayed on the label by the manufacturer.

Studies completed thus far are very encouraging. This method may offer a standard objective estimation of potency with many advantages over assay now being used for commercial long-acting ACTH preparations.

The Effect of Reserpine on Pituitary-Adrenal Function

By *John A. Anderson.* Department of Pediatrics, University of Minnesota Medical School, Minneapolis.

A study on the effect of Reserpine on the pituitary-adrenal mechanism has been made in

children and in normal and hypophysectomysed monkeys and dogs. Changes in the circulating eosinophils, the excretion of 17-hydroxycorticoids, the venous blood glucose and steroid discharge responses to ACTH before, during, and after reserpine administration have been observed.

Reserpine-treated children appear to have a greater response to a test ACTH dose. The daily renal excretion values of 17-hydroxycorticoids did not appear to be significantly increased. Reserpine appeared to produce a striking eosinopenia in normal monkeys and in dogs but not in the hypophysectomysed animal. This eosinopenic effect could be demonstrated during the a.m. to p.m. period, as well as for the p.m. to a.m. period. The excretion of 17-ketosteroids was increased in 3 monkeys maintained on Reserpine for a period of 9 days.

These studies suggest that reserpine is able to activate the ACTH-steroid release mechanism. The daily rhythm of eosinophils in the blood appears to be abolished by Reserpine medication. It has been demonstrated by others that Reserpine releases 5-hydroxytryptamine and related substances in the brains of animals. A relationship between serotonin and serotoninlike substances to the rhythmic functioning of the ACTH-adrenal mechanism is suggested.

In Vitro Stimulation of Rat Adrenocortical Secretion by Corticotropin and a Protein Factor from Human Urine

By *Patrick J. Mulrow, Amos H. Lieberman, George L. Shmagranoff and John A. Luetscher, Jr.* Stanford University School of Medicine, San Francisco.

Rat adrenal glands incubated in Krebs-Ringer-Bicarbonate solution in an atmosphere of 95% O_2, 5% CO_2, secrete at least four compounds that can be separated by paper chromatography. Compound I, a substance more polar than hydrocortisone, does not reduce blue tetrazolium but has a peak ultraviolet light absorption at 243. Compound II, eluted from the cortisone region of the paper chromatogram, has sodium-retaining activity by bio-assay and is believed to be aldosterone. Compound III, slightly more polar than corticosterone, does not reduce blue tetrazolium but has a peak ultraviolet light absorption at 240. Compound IV has been tentatively identified as corticosterone.

Addition of commercial lyophilized corticotropin to the adrenal incubate increased the output of all compounds, including aldosterone. Corticosterone represented the major fraction of the steroid increment.

The addition of the protein obtained from human urine by ultrafiltration through a collodion membrane increased the secretion of aldosterone. Its effect on the other three compounds was not as marked as that of corticotropin. Prolonged boiling destroyed the activity of urine protein.

These observations suggest that selective secretion of a single corticosteroid or group of corticosteroids may be stimulated by different protein mixtures. The isolated rat adrenal offers an approach to the study of this problem.

A Criterion for the Choice of Bilateral Total vs. Subtotal Adrenalectomy in Cushing's Syndrome

By *V. DiRaimondo, S. Hane and Peter H. Forsham.* Metabolic Unit and the Endocrine Clinics of the Department of Medicine, University of California School of Medicine, San Francisco.

Decision between the above-mentioned alternatives might logically be based on whether or not there will be a functional restitution of the original secretory capacity of both adrenal cortices after removal of one or more. This problem has been subjected to analysis in three endocrinologically normal subjects and eight patients with Cushing's syndrome due to bilateral adrenal cortical hyperplasia. Basal outputs of 17-hydroxycorticoids and 17-ketosteroids were followed and, in addition, the steroidogenic responses of the adrenals to maximal stimulation with ACTH was evaluated by an 8-hour intravenous ACTH test both before and after subtotal adrenalectomy. The basal outputs of steroids rapidly increased to preoperative levels in both normals and in all patients with Cushing's syndrome. The steroidogenic capacity, however, was found to remain halved for up to two years in all but two of the cases with Cushing's syndrome who showed a return to the original steroidogenic capacity within one month after unilateral adrenalectomy. Normal male guinea pigs, in contrast to humans, were found to behave exactly like these two exceptional patients, regaining 100% of steroidogenic capacity with an increase in gland weight of only 15% after unilateral adrenalectomy. By demonstrating a return to the preoperative steroidogenic capacity within one month after unilateral adrenalectomy, a criterion for total bilateral adrenalectomy in man might be established.

Periodic Paralysis Associated with Hyperthyroidism and the Role of Aldosterone in the Pathogenesis

By *Milton G. Crane.* Department of Internal Medicine, College of Medical Evangelists, Los Angeles. (Aided by a grant from the National Institutes of Health, U.S.P.H.S.)

Recent developments in the study of mineralocorticoids have suggested that aldosterone might play a key role in the pathogenesis of periodic paralysis whether of the familial type or the type associated with hyperthyroidism.

A 23-year-old white male with periodic paralysis was observed in several paralytic episodes. The 24-hour radioiodine uptake test and P.B.I. were 71% and 13.1 μg., respectively. The exchangeable sodium using Na^{22} was 43.5 mEq. per Kg. (normal 37.8–47.5). The exchangeable potassium, using K^{42}, was 44.0 mEq. per Kg. (normal 39.8–63.3). During the attacks the serum potassium was found as low as 2.1 mEq./L. An aldosterone assay was performed (courtesy of Dr. Jerone Conn) on two 24-hour samples of urine obtained during an attack of paralysis and were 25 gamma per day (normal 1–3).

Conn has studied a patient with familial periodic paralysis and finds that during an attack of paralysis there is a high output of aldosterone. It is concluded that the pathologic physiology of the periodic paralysis associated with hyperthyroidism is as follows: there is an inborn latent physiologic defect in the adrenal cortex which, under the conditions of hyperthyroidism and stress, results in an intermittently high secretion rate of aldosterone. The clinical manifestations can then be explained by the influence of aldosterone upon mineral metabolism, particularly potassium.

17-Hydroxycorticoid Excretion With and Without Stimulation in Diabetics

By *Elmer A. Anderson, Benjamin Cutshall, Jr. and William W. Pote, Jr.* College of Medical Evangelists, Los Angeles.

17-hydroxycorticoid excretion was studied in diabetics with various complications, with and without stimulation, in order to determine if there is a characteristic pattern.

On each patient a 24-hour collection of urine was made and 17-hydroxycorticoid level was determined by the Brown modification of the Reddy and Thorin method. The groups of patients studied were in the following categories: (1) Diabetic acidosis; (2) Postacidosis with or without diabetic complications; (3) Patients with triopathy; (4) Varied medical problems not necessarily diabetic.

In some patients in all four groups, the 17-hydroxycorticoid excretion was studied with or without subsequent stimulation of the adrenal. In groups 2 to 4, most of the patients had one control determination and two determinations with stimulation. For stimulation, 25 U. S. P. units of ACTH was given by 8-hour intravenous infusions on two successive days.

The values of 17-hydroxycorticoid excretion obtained in the 55 control determinations ranged from 0 to 48 mg. Twenty-five patients showed values of 5 mg. or less. All patients in acidosis showed values above 20 mg. Some in the early postacidotic period showed elevated levels while others had returned to normal within 48 hours. Several patients with triopathy had very low values. There was no pattern in group 4.

The results of stimulation were as follows: Postacidotic patients: 2.3 mg.–63.2 mg.; Triopathy patients: 0.76 mg.–20.6 mg.; Patients in group 4: 3.8 mg.–52.6 mg.

17-hydroxycorticoid excretion in diabetic patients with varied general medical complications shows no characteristic pattern. However, the acidotic patients show high values and triopathy patients tend to show low values. The adrenal stress of acidosis appears comparable to the stimulation of 25 U.S.P. units of ACTH. Most triopathy patients were responsive to stimulation. The initial level of 17-hydroxycorticoid excretion in triopathy and the general lower response levels suggest decreased adrenal cortical function when measured by this method.

Clinical Experiences Following the Oral Administration of Orinase in Nondiabetic and Diabetic Humans

By *Thomas H. Lambert, William F. Bethard, Solon Palmer, Jr., and Lee S. Monroe.* Scripps Clinic and Research Foundation, La Jolla, California.

The effect of Orinase was observed in 8 nondiabetic and in 37 diabetic patients who needed insulin for control. The 8 nondiabetic patients were hospitalized. During the period of observation they remained in bed and their diets were constant. A glucose tolerance test, CBC, and urinalysis were done the first day of admission. Blood glucose determinations were made at 15- to 60-minute intervals for 8 hours the day preceding the oral administration of Orinase, the day of administration of Orinase, and the day after Orinase was given. The 37 diabetic patients, including 27 patients on insulin and 10 patients (one of whom was juvenile) not on insulin, were hospitalized from 2 to 4 weeks, and an attempt was made to control the diabetes with Orinase or Orinase in combination with insulin. Prior to therapy and at intervals following initiation of therapy, these patients had chest x-ray, EKG, CBC, PBI, I^{131} uptake, urinalysis, IVPSP, blood urea, BSP and thymol turbidity. None of these tests was found to be abnormal. The only serious manifestation observed was a skin rash in two patients. In the nondiabetic group the maximum blood sugar lowering effect was noted within one to two hours after oral administration of Orinase. The diabetics who were taking large doses of insulin developed hyperglycemia, ketosis, and increased excretion of glucose in urine when Orinase was substituted for insulin. Orinase was found to be successful in 16 mild, stable diabetics within the 44–70 age group, with an insulin requirement of 5–30 units per day, and whose weight was normal. The duration of the disease prior to treatment, and the duration of insulin therapy before begin-

ning Orinase did not seem to influence greatly the response to the drug.

Experiments Indicating That the Levator Ani Muscle Method of Assay is Not Suitable for the Screening of Protein Anabolic Steroids

By *E. Geiger and M. Nimni.* Departments of Pharmacology and Biochemistry, University of Southern California Medical School, Los Angeles.

Experiments were conducted to determine whether the growth of the musculus levator ani of male rats induced by the injection of steroid compounds is a valid indicator of protein anabolic activity, as claimed by several investigators.

Wistar male rats were employed in the present study. In the preparation of the levator ani, the procedure of Hershberger et al. was followed. In the respective experiments, the following substances were injected subcutaneously daily: (1) Testosterone propionate (Lilly), 3 mg. for 5 days; (2) Norethandrolone (Nilevar-Searle), 3–5 mg. for 5 days; (3) Pit. Gonadotropic Hormone (Squibb), 2 units for 10 days; (4) Antuitrin-Growth (Parke-Davis), 100 units for 10 days.

The following results were obtained: (1) After castration, the levator ani of adult male rats lost about 60–70% of its weight. In contrast, the total body weight and the weight of the skeletal muscles decreased insignificantly. (2) In immature rats on a protein-free diet, not only was growth stunted but the weights of the levator ani, the testes, the seminal vesicles and the kidneys were also abnormally small. When testosterone was injected during the depletion period, the weights of the levator ani, seminal vesicles and kidneys increased significantly, in spite of the protein-free diet. The total body weight and the weight of the diaphragm muscle were not influenced by the hormone injections; they were similar to those of non-injected controls. (3) Similar results were observed in castrated adult rats fed a protein-free diet. When Norethandrolone was injected, comparable results were also obtained. (4) Injection of gonadotropic hormone to normal rats on protein-free diets increased the growth of the levator ani and of seminal vesicles. The injection of growth hormone was ineffective. In castrated rats on protein-free diet, the injection of gonadotropic hormone did not stimulate growth of the levator ani and of seminal vesicles.

These results suggest that when the growth of the levator ani is stimulated by hormone treatment on a protein-free diet, the proteins necessary for the growth of these organs are derived from other tissues, possibly the liver or skeletal muscles. These findings indicate that the hormone-induced anabolism in the levator ani is associated with protein catabolism in other organs.

It is concluded that the growth of the musculus levator ani is not a suitable method for determining the anabolic properties of steroid compounds.

The Effect of Testosterone Therapy in Eighty-Seven Cases of Male Infertility

By *W. M. Laidlaw, R. S. Tether and C. G. Heller.* University of Oregon Medical School, Portland, Oregon.

Diminution of seminiferous tubular sclerosis was noted as an incidental finding during studies which showed that damage to the normal human testis by testosterone was reversible. Studies were then undertaken to evaluate this effect as a possible means of therapy in the infertile male.

The subjects were 87 infertile males who, by testicular biopsy, showed either varying degrees of tubular sclerosis and disorganization and sloughing of the germinal elements or partial to complete germinal cell arrest. The subjects' wives had previously been evaluated and were considered to be fertile. Urinary gonadotrophin assays were performed before and, in some cases, during and after, therapy. Base-line sperm counts were obtained prior to therapy and every two weeks thereafter for a minimum of one year. Most of the subjects received a total of 2000 mg. of testosterone propionate in oil, although a few received as much as 4000 mg.

To date 41 of the 87 subjects have maintained a higher sperm count than before therapy. No change in the sperm count was observed in 27 subjects; 19 now have counts significantly lower than before therapy. In some cases the quality of sperm motility was improved but this was not a uniform observation. The wives of 26 of the subjects have become pregnant during the study. Testosterone therapy did not influence the ejaculate volume and apparently did not effect the percentage of abnormal sperm produced.

The pretreatment testicular biopsy, gonadotrophin assay, and routine sperm count were found to be essential in selecting the proper patient for this mode of therapy. A greater number of pregnancies occurred within this group than has been previously observed by us in a similar population.

"Medical D & C" with Single Injections of 17-alpha-hydroxyprogesterone-n-caproate

By *Felix O. Kolb.* Metabolic Unit and the Department of Medicine, University of California School of Medicine, San Francisco.

Administration of progesterone to produce withdrawal bleeding in states of anovulation with a proliferative endometrium, the so-called "medical D & C" of Albright, has had diagnostic and therapeutic use. The irregular action of orally administered progesterones and the inconvenience of daily

injections of progesterone in oil has been a common experience. The recent introduction of 17-alpha-hydroxyprogesterone-n-caproate (Delalutin, Squibb) has provided a long-acting progesterone analog that has shown excellent progestational activity in animal experiments. This compound was administered as an oily solution containing 125 mg./cc. intramuscularly as a single injection of 2 cc. to a series of some 20 women. Some were in states of anovulation, varying from temporary amenorrhea, lasting several months, to over 10 years, and others showed mild to moderate dysfunctional bleeding. In every instance where adequate proliferative endometrium was present, withdrawal bleeding, preceded by varying degrees of basal body temperature rises and subsequent falls, occurred within 8 to 17 days, with an average of 12 days. Short-acting progesterone compounds, by contrast, would produce withdrawal bleeding within 2 days of stopping the medication. In many cases, regular ovulatory cycles for several months to over a year were established by single injections of 250 mg. of Delalutin; other patients required two or more cycles. The relatively slow initial action of this compound has made it less effective in stopping profuse dysfunctional bleeding. Aside from tenderness at the site of injection, breast tenderness, and occasional nausea, no side-effects were observed. Withdrawal bleeding was not seen in instances of complete ovarian failure, as in premature menopause and in amenorrhea due to pregnancy or to adrenal androgenic overactivity. The combination of an estrogenic compound with Delalutin in such cases is under investigation.

Ovarian Polycystosis: Successful Nonsurgical Management with Psychotherapy Plus X-Ray Therapy Aided by Transvaginal Pelvioscopy

By *A. R. Abarbanel.* Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles.

That gonadal function may be disturbed by psychosomatic stress is common knowledge. This report presents a simplified, nonsurgical method of managing one of these clinical aberrations of ovarian function—the syndrome of ovarian polycystosis.

By means of transvaginal pelvioscopy, direct visualization established the diagnosis of ovarian polycystosis in 54 women. For clinical purposes, three functional types were recognized:

Type I. Parvi-polycystosis, 28 cases; Type II. Macro-polycystosis, 23 cases; Type III. Sclero-macro-polycystosis, 3 cases.

Of these 54 cases, 23 were subjected to a limited form of group psychotherapy with 6, or 26%, establishing regular menses and subsequent pregnancy. In the remaining 48 cases, "x-ray stimulating therapy" was given according to the technic of Kaplan. Thirty-eight, or 79.2%, established regular menses with subsequent conception.

It was observed that where the tunica was thin over the follicles, therapeutic response was usually excellent, whereas when the tunica was universally sclerotic (3 cases), surgery aided restoration of menses in two, but neither conceived.

In summary, 54 women with varying degrees of ovarian polycystosis were studied. With limited psychotherapy plus so-called "x-ray stimulation therapy," regular menses and subsequent conception occurred in 81.5%. These results are almost identical to those obtained by surgical intervention.

The Effect of Ovariectomy and Fetectomy on Trophoblastic Proliferation

By *Robert W. Noyes and Zeev Dickmann.* Department of Obstetrics and Gynecology, Stanford University School of Medicine, San Francisco.

Trophoblast cells, particularly trophoblastic giant cells, resemble cancer in that they are able to penetrate endometrium and myometrium and to metastasize widely in the body. Little is known about the dynamics of trophoblastic proliferation and maternal resistance to invasion, but evidence reported in the literature suggests that both the embryo and the ovary play important roles. The purpose of this study is to estimate quantitatively the effect of trophoblastic proliferation of ovariectomy, fetectomy, and both together during midpregnancy in the rat.

Four groups of rats were operated upon on the twelfth day after mating. In a control group, a shallow incision was made in the uterus, a second group was ovariectomized, in a third group several embryos were removed, the placentas remaining, and in a fourth group both ovaries and some of the embryos were removed. Two days later the animals were killed and several embryonic sacs from each were fixed in formalin, serially sectioned, and stained. The volume of the reticularis (small trophoblast cells) was obtained by tracing projected sections, cutting out the silhouette, and weighing the pieces of paper. The giant trophoblast cells were counted. Fetectomy alone caused a shrinkage of the sac and placenta which, in turn, caused an apparent increase in the area of reticularis and giant cells in a given section. Volumetric analysis, however, showed that this apparent increase was an artefact. Ovariectomy caused little, if any, proliferation of trophoblast when the embryo was present. In the ovariectomized fetectomized animals, the number of giant cells is increased, though not reticular cells. It is possible that both the embryo and the ovary play a role in restraining giant cell proliferation. At least, the data are worthy of further amplification.

Increase in Pregnanediol Titer Following Administration of Mammotrophin

By *Robert A. Lyon and B. P. Lelich.* Children's Hospital, San Francisco, and the Kaiser Foundation Hospital, San Francisco.

Prior studies have shown increase in urinary pregnanediol output when large doses of progesterone have been given and also following administration of moderate doses of chorionic gonadotrophin. This report indicated that pregnanediol titers were increased significantly when mammotrophin (sheep) was administered, provided it was given in adequate dosage at precise phases of the human ovarian cycle. The intent of this report was to investigate the luteotrophic activity of pituitary mammotrophin as reflected by pregnanediol output (method of Somerville, Gough, and Marrian). The doses of mammotrophin employed were considerably higher than those reported by other observers. Pregnanediol levels exceeded the control level from two- to three-fold in each ovular woman but not in nonovulated control patients. This supports the concept that exogenous mammotrophin is luteotrophic in man. Metabolic evaluation of treated patients has shown no contraindication to its use. Antihormone has not been demonstrated.

The Effect of Mobilization on Hypercalciuria Following Acute Poliomyelitis

By *Fred Plum and Marcelle F. Dunning.* Department of Medicine, Division of Neurology, University of Washington School of Medicine, Seattle.

A previous study from this laboratory demonstrated that hypercalciuria invariably follows paralytic poliomyelitis. In the present study, the effect of body mobilization on this hypercalciuria was analyzed in 45 subjects with varying degrees of paralysis.

All subjects were on a daily calcium intake of either 0.5 Gm. or 0.8 Gm. Twenty-four hour urine samples were pooled in 4-day collection periods each week. Urine calcium, phosphorus and creatinine were analyzed by standard methods. Serial analyses were continued for several months in each subject. Active and passive exercises, sitting, standing, crutch-walking and independent walking were introduced at selected intervals after onset of poliomyelitis. The time between onset of disease and the initiation of activity differed widely in patients with comparable distribution and degree of paralysis. It was possible, therefore, by comparing excretion patterns in different patients, to determine both the immediate and long-term effect of mobilization on hypercalciuria.

In no instance did any of the mobilization procedures, short of walking, exert any immediate ameliorating effect on hypercalciuria. Indeed, many subjects showed transient increases in urinary calcium loss following one or more mobilization procedures. In groups of patients with comparable degrees of paralysis, no significant difference in the duration of hypercalciuria was observed between those mobilized early and those mobilized late. The effects of walking were equivocal: of 11 patients who walked with crutches, 3 had normal calcium excretion prior to walking, 4 showed increased calcium loss associated with walking, and 4 maintained persistent hypercalciuria for at least several weeks after walking began.

It is concluded that the duration of hypercalciuria following poliomyelitis depends on the amount of muscle tissue denervated; artificial mobilization procedures have little effect in minimizing or shortening the duration of mineral loss.

The Labeling of Serum Albumin for Turnover Studies

By *Sheldon Margen and Harold Tarver.* Department of Physiologic Chemistry, University of California School of Medicine, Berkeley. (Aided by a Grant from the Department of Health, Education and Welfare.)

Previous reports from our laboratory have demonstrated marked differences in turnover rates of serum albumin depending upon the method of measurement employed. The *standard of reference* involves the use of biologically synthesized albumin-S^{35}. Measured against this reference albumin-I^{131} gave shorter half-lives, and the labeling by injection of methionine or cystine-S^{35} gave much longer half-lives.

Because of the difficulties in preparing endogenously-labeled albumin and in an endeavor to find a technic that would correspond to the *standard of reference*, we have turned to a method of labeling with amino acids, the carbaminoanhydride method. This procedure leads to the addition of the labeled amino acid, in this case methionine-S^{35}, to the free amino groups of the protein, either the epsilon amino groups of lysine or the terminal amino groups of the protein chains. The protein is not denatured in the process; in zone electrophoresis on paper it behaves as the original material.

Using this method, we have labeled human serum albumin with methionine-S^{35}, or with both methionine-S^{35} and I^{131}. When the degradation of the protein was studied in human subjects it was found that the half-life of the S^{35} label was similar to that observed when the "standard" method was employed. Using the doubly-labeled material, the I^{131} was found to be lost much more rapidly than the S^{35}, that is, the apparent turnover rates were different depending upon the label observed.

We conclude that at least part of the I^{131} is lost from the protein by a process which does *not* involve protein degradation. Hence the labeling of proteins in this manner may lead to erroneous conclusions

with regard to turnover rates or other metabolic processes.

Study of the Essential Amino Acid Requirements of Men Over Fifty

By *Stewart G. Tuttle, Marian E. Swendseid, Wendell H. Griffith, and Samuel H. Bassett.* Research Service, V. A. Center, Los Angeles; and the Department of Medicine, Physiological Chemistry and Home Economics, University of California Medical Center, Los Angeles. (Aided by a grant from Nutrition Foundation, Inc.)

The minimum requirements of the eight essential amino acids necessary for the maintenance of nitrogen equilibrium have been tentatively established by a number of investigators, using young adults as subjects. The present study was undertaken to determine whether males over 50 have the same requirements. Five healthy men ranging in age from 52 to 68 years were placed on control diets, the caloric intake of which was varied according to the individual requirements of each subject, but whose protein content and sources were kept constant at 43.7 Gm. (7 Gm. nitrogen). After a suitable control period (usually 12 days), during which it was ascertained that the patient remained in nitrogen equilibrium or slightly positive balance, he was given essential 1-amino acids in the proportion found in egg protein and in quantities equal to or exceeding the levels at which all younger subjects maintained nitrogen equilibrium. Glycine was added to the diet to bring the daily nitrogen intake to 7 Gm. Without exception, all subjects went into negative nitrogen balance. In three individuals, after reestablishment of equilibrium by feeding the control diet, the protein of the former was replaced by natural egg protein in a quantity sufficient to provide identical amounts of the essential amino acids used in the first experimental diet. In two of the three subjects this natural source of amino acids failed to maintain nitrogen balance. Only when the amounts of the essential amino acids were doubled was nitrogen equilibrium maintained. The occurrence of a negative nitrogen balance in these older subjects when receiving quantities of amino acids sufficient to maintain all previously studied young adults, and substantially in excess of the amounts needed by most of them to maintain nitrogen equilibrium, suggests that the quantitative requirements for one or more of the essential amino acids are increased in older men.

Essential Fatty Acids and Lipid Metabolism: Further Observations

By *Laurance W. Kinsell, George D. Michaels, H. P. Chin, George Fukayama, Robert A. Peacock and Stanley J. Talpers.* Institute for Metabolic Research of the Highland County Hospital, Oakland, California.

An increasing body of evidence confirms the concept that the relative or absolute essential fatty acid content of a diet is related to the level of plasma cholesterol and phospholipid, and may be related, therefore, to the over-all problem of atherosclerosis.

The evaluation of the role of essential fatty acids in lipid metabolism has been under constant investigation in this laboratory. Among the substances used in studies on patients in the metabolic ward are (a) ethyl esters of pure fatty acids; (b) ethyl esters of mixtures of fatty acids derived from natural fat; and (c) synthetic triglycerides.

On the basis of such studies it has been demonstrated that change of the nature of the fatty acid intake can significantly modify the fatty acids that are esterified with plasma cholesterol, phospholipids and neutral fat. The data so far available are compatible with the concept that at least a portion of the mechanism of the effects of essential fatty acid on lipid metabolism is by way of increased efficiency of fatty acid transport, and that this in turn results in diminished endogenous production of cholesterol and phospholipids.

Acute Effects of Fat Ingestion, Carbohydrate Ingestion, and Fasting on the Concentrations of Chemical Constituents of Human Serum Lipoproteins

By *Richard J. Havel.* Laboratory of Metabolism, National Heart Institute, Bethesda.

This study was undertaken to provide information regarding the participation of constituents of serum lipoprotein fractions in fatty acid transport. Lipoproteins were separated in the preparative ultracentrifuge at increasing solvent densities to yield three fractions of densities: <1.019 (I + II), 1.019–1.063 (III), and 1.063–1.21 (IV). Chemical analyses for several lipid constituents and protein were performed on each fraction. Ingestion of 1.5 Gm. fat/Kg. body weight in healthy adults resulted in increases in cholesterol, phospholipids, and glycerides in fraction I + II. No significant alteration occurred in fraction III, but phospholipid concentration in fraction IV increased regularly, and cholesterol and protein concentrations increased occasionally. The fall in phospholipid concentration in this fraction lagged behind the fall in fraction I + II constituents. The effects observed occurred after ingestion of cream, corn oil, and eggs plus oleomargarine. Effects of fasting and glucose ingestion were studied in seven healthy adults. Each period of study followed a 12–15-hour fast to minimize chylomicronemia. Concentrations of all lipid constituents of fraction I + II were variable during continued fasting, but fell consistently after ingestion of 400 Gm. glucose over a four hour period (mean changes after eight hours: +1% fasting and −43% glucose). No significant change in fraction III constituents occurred during either period.

Cholesterol and protein concentrations in fraction IV did not change, but phospholipid concentration rose slightly during continued fasting and fell significantly following glucose. These findings permit only tentative conclusions. While increased concentrations of fraction I + II constituents after fat ingestion represent principally chylomicron transport, alterations observed following glucose ingestion as opposed to fasting indicate that this fraction may also participate in fatty acid transport during fasting. Alterations in fraction IV phospholipid concentration observed during these studies suggest a role in both exogenous and endogenous fatty acid transport.

The Mechanism of Triton-Induced Hypercholesteremia

By *Meyer Friedman and Sanford O. Byers.* Harold Brunn Institute for Cardiovascular Research, San Francisco.

The mechanism by which injection of the detergent Triton induces hypercholesteremia has been investigated.

Following the injection of Triton, we studied (1) the rise and subsequent falls in plasma cholesterol, phospholipid and triglyceride; (2) the hepatic content of cholesterol; and (3) the change in hepatic lymph content of cholesterol. Similar studies were done after experimental alterations in the hepatic rate of synthesis of cholesterol. Finally, the effects of Triton upon the plasma of the hepatectomized animal under various conditions were studied.

It was observed that following injection of Triton, a rise of plasma neutral fat occurred first, to be followed in turn by cholesterol and phospholipid. The hepatic content of cholesterol could not be increased in the Triton-injected animal despite the ensuing hypercholesteremia, nor could the cholesterol content of hepatic lymph be elevated during the period of developing hypercholesteremia. The hypercholesteremic effect of Triton also was found to induce a marked hypercholesteremia in the liverless animal if either neutral fat or phosphatide were also administered.

These results suggest that the injection of Triton induces hypercholesteremia in the following manner. First, this detergent leads to the sequestration and accumulation in plasma of both neutral fat and phospholipid. The excess plasma neutral fat and phospholipid in turn extract from and hence mobilize cholesterol, not only from hepatic but also from extrahepatic stores of the body. Accordingly, the intensity of this hypercholesteremia produced by Triton is dependent upon (1) the degree of "hypertriglyceridemia" and hyperphospholipidemia initially evoked by the circulating Triton and (2) the available body stores of cholesterol, particularly the extrahepatic ones. These conclusions offer further evidence for the concept that various types of hypercholesteremia may be induced by an initial elevation of "hypertriglyceridemia," hyperphospholipidemia, or both.

GASTROINTESTINAL SYSTEM

The Effects of Variations in Potassium Concentration on Ion Transport and Bioelectric Potentials Across the Frog Gastric Mucosa

By *John B. Harris, H. Frank and I. S. Edelman.* Department of Medicine, University of California School of Medicine, San Francisco.

Both the distal renal tubular cells and the parietal cells of the gastric mucosa secrete hydrogen ions and maintain a negative intraluminal potential, as compared with the surrounding interstitial fluid. The purpose of this study was to examine the possibility that both tissues may have similar ion transport mechanisms.

The transport of Na^+, K^+, Cl^-, and H^+ volume production and bioelectric potential of the gastric mucosa of *Rana pipiens* was measured in vitro, using the Durbin-Frank-Solomon chamber method. The serosal side of the mucosa was bathed in buffered modified Krebs-Henseleit solutions containing 0.9, 3.2 or 8.5 mM/L. of KCl. Fourteen experiments have been completed to date. Net Na^+ and Cl^+ flux from nutrient to secretory tended to be greatest with low K^+ nutrients, but more data are needed to prove this effect. Net K^+ flux into the secretory phase did increase significantly with a high K^+ medium averaging 1.1 μEq./hour higher than the control value of 1.3 μEq./hour per 1.6 cm^2. Acidification of the secretory phase was not inhibited by increasing K^+ concentrations, since H^+ production averaged 0.3 μ/Eq./hour more with high K^+ as compared to low K^+ in the nutrient. Volume production tended to be least with a high K^+ nutrient and greatest with a low K^+ nutrient. The transmembrane potential was inversely proportional to the nutrient K^+ concentration, the average figures being -37 ± 5 mV, -26 ± 7 mV and -22 ± 5 mV with K^+ concentrations of 0.9, 3.2 and 8.5 mEq./L., respectively.

These data indicate fundamental differences in the ion transport mechanisms of renal tubular and gastric mucosal cells, since high K^+ loads to the kidney result in a diuresis of Na^+, Cl^- and H_2O and inhibition of H^+ secretion.

Changes in Gastric Acidity, Uropepsin and 17-Hydroxycorticoid Excretion after X-ray Therapy to the Stomach for Treatment of Peptic Ulcer

By *J. A. Rider, H. C. Moeller and T. L. Althausen.* Gastrointestinal Clinic, Department of Medicine, University of California School of Medicine, San Francisco.

In selected cases, x-ray therapy to the stomach has become an accepted form of treatment for both gastric and duodenal ulcer. It is especially valuable for the older patient and for those in whom medical complications have increased the surgical risk.

A tissue-depth dose of 1500 r produces optimal results. A significant reduction in gastric acidity coincident with clinical improvement was observed in a majority of the patients so treated.

Our previous work, as well as that of others, has shown a positive correlation between gastric acidity and urinary uropepsin excretion. However, during and immediately after x-ray therapy to the stomach, a paradoxical inverse relationship was observed between gastric acidity on the one hand and excretion of urinary uropepsin and 17-hydroxycorticoids on the other. This inverse relationship became apparent during and immediately after exposure to radiation therapy, during which time the usual reduction in gastric acidity occurred. This inverse relationship persisted from 1 to 3 months. This phenomenon was not observed in a small group of patients in whom x-ray therapy failed to depress gastric acidity.

Our results suggest physiologic mechanisms that may have therapeutic implications.

The Effect of Time, Temperature and pH on the Accuracy of Uropepsin Determination

By *Peter Stathakos, Konstantin Arensburger and Leonard Molofsky,* Oakland, California.

Previous workers have stressed the need for using fresh refrigerated 24-hour specimens of urine in the determination of uropepsin concentration, but no definite restriction of the time the urine may stand after collection has been delineated. During a study of the effect of pregnancy on uropepsin excretion, the need arose to establish strict criteria of time and temperature for standing of the specimens.

Determinations of uropepsin were made in 14 patients, using the method of Gray et al., on fresh urine specimens, and repeated after standing 2, 3, 5 and 7 days. Specimens were divided in half, one portion standing at room temperature, and the other under refrigeration at 4.5°C.

After the first day, a gradual increase in the development of chromogenic substances was noted, with resultant increase in the uropepsin values. The greatest increase occurred after the third day. These changes were considerably less manifest in the refrigerated specimen. The rate of change appeared unrelated to the initial urinary pH.

Conclusion: Uropepsin determination on unrefrigerated 24-hour urine specimens must be performed within 24 hours of collection. On refrigerated specimens, determination may be delayed for no longer than 3 days.

The Reaction of Viral Hepatitis Sera with M. Rhesus Erythrocytes

By *Robert E. Hoyt and Lester M. Morrison,* Los Angeles.

At present, no specific method of diagnosis or screening for virus hepatitis is available. Results are reported on a hemagglutination reaction obtained when the blood serum of patients with viral hepatitis of both infectious hepatitis (IH) and homologous serum hepatitis (SH) strains acts against M. rhesus erythrocytes.

The agglutination titer (HAT) was found to be under 1:8 in 375 out of 431 patients with various medical and surgical illnesses, exclusive of jaundice or viral hepatitis. It was thus negative in 86% of "controls." The HAT was positive in 56 cases (14%). The HAT was found to be positive (1:8 or higher) in 19 (86%) of 22 acutely ill patients suffering from acute virus hepatitis. Three of these patients (14%) gave negative results. The test was found to give positive results in 10 of 14 patients with the chronic form of virus hepatitis who had signs or symptoms of the disease up to 2½ years after its acute onset. Ten of these (71%) gave a positive test, whereas 4 (29%) gave negative results. The HAT was consistently valuable in giving clear-cut negative results in nonviral hepatitis, jaundiced patients. Of 21 patients, all gave negative reactions.

These results suggest further studies to determine the practical possibilities of this test in clinical practice and as a possible screening procedure for blood transfusion donors.

Catheterization of the Portal Vein Through a Portacaval Anastomosis in Patients with Cirrhosis

By *Telfer B. Reynolds, Herman M. Geller and Alan G. Redeker.* Department of Medicine, University of Southern California School of Medicine and the Los Angeles County Hospital, Los Angeles. (Aided by a grant from the National Institutes of Health.)

By an approach from an antecubital vein and the inferior vena cava it has been possible to catheterize the portal vein in 5 cirrhotic patients who had previously had an end-to-side portacaval anastomosis performed. Differentiation of the portal vein from the left renal vein was achieved by angiography or by infusing para-aminohippurate and

noting the degree of extraction of this substance in the vein in question. Measurements were made without the use of any premedication or anesthesia. Patients were in the fasting state.

Pressure levels in the portal vein ranged from 2 to 7 (mean 4.2) mm.Hg. above inferior vena cava pressure, attesting to the efficacy of the shunt in relieving portal hypertension. Pre- and postshunt portal vein pressures, determined at surgery, averaged 26 and 11 mm.Hg respectively in the same patients.

In 10 paired simultaneous samples portal venous oxygen content averaged 1.41 vol. % below arterial oxygen content. The portal stream is, therefore, an important source of hepatic oxygen supply in the fasting state. Portal venous whole blood CO_2 content averaged 0.85 vol. % higher than arterial CO_2 content in 7 comparisons.

During a continuous infusion of bromsulphthalein (BSP), levels of this substance in the portal venous and arterial blood did not differ significantly in 11 simultaneous comparisons. One of the potential sources of error in the BSP infusion method of measuring hepatic blood flow is extrahepatic metabolism of BSP. The lack of difference between arterial and portal venous BSP concentrations suggests that the extrahepatic splanchnic area does not remove BSP from the blood.

Blood Amino Acid Patterns in Hepatic Coma

By *Sherman M. Mellinkoff, Marjorie Frankland, Margaret Greipel and Henry Shibata.* Department of Medicine, University of California Medical School and the Wadsworth V. A. Center, Los Angeles.

Only complete or nearly complete destruction of the liver appreciably changes the total amino-acid concentration in the blood. The concentration of individual amino acids, however, may be abnormal in less severe hepatic disease. The blood amino-acid pattern was investigated in Laennec's cirrhosis, hepatitis, and hepatic coma, to see if a single type of abnormality was constant in the latter. Heparinized venous blood from individuals in the fasting state was deproteinized with absolute alcohol. Twenty-five microliter aliquots were spotted on filter paper and two-dimensional chromatograms were made with butyl alcohol and phenol solvents. Color photographs were taken after spraying with 0.2% ninhydrin. Variations in pattern were explored in 30 normal individuals. Nine of these individuals were tested a total of 28 times, revealing remarkable day-to-day constancy in the same individual. Twenty-six patients with Laennec's cirrhosis and 14 with viral hepatitis, none in coma, were tested. In most of them, individual amino acid concentrations were not clearly abnormal by this technic, and in none was any abnormality very marked. Seven patients in hepatic coma all had obviously abnormal amino-acid patterns, but no two of them were abnormal in the same way. Abnormalities included, for example, a greatly increased concentration of leucine in one patient and virtual absence of leucine in another. A number of chromatographic spots were found that were never seen in the normal and noncoma groups. Most of these spots remain unidentified. One was identified as guanido-acetic acid, suggesting failure of hepatic methylation, of this compound, in only one patient. One patient thought to be in hepatic coma had a normal chromatograph and was subsequently found to have had a cerebro-vascular accident. The following conclusions are reached: (1) amino acid chromatography may be useful in the diagnosis of hepatic coma; and (2) hepatic coma may be accompanied by various divergent defects in protein metabolism.

The Influence of Parabiosis on the Synthesis of Liver Nucleic Acids after Hepatectomy

By *J. L. Van Lancker and J. H. Maisin.* Department of Pathology, University of Utah, and the Cancer Institute of the University of Louvain (Belgium).

Partial hepatectomy of one parabiotic rat stimulates mitosis in the liver of the nonoperated partner (Bucher et al.). In the present study the incorporation of C^{14}-labeled orotic acid, a specific pyrimidine precursor, into the DNA of the livers of both parabiotic partners has been measured at 36 and 72 hours after ⅔ hepatectomy of one partner, as an index of nucleic acid synthesis. The incorporation into the nuclear and cytoplasmic RNA was measured at 36 hours after hepatectomy. Parabiosis was established two months before hepatectomy. The orotic acid was injected intraperitoneally 24 hours after operation. The cellular particles and nucleic acids were separated as described by Hurlbert and Potter. In normal rats there is no significant incorporation of orotic acid into liver DNA but it is markedly elevated after hepatectomy. Hepatectomy of one parabiont stimulates the orotic acid incorporation in the liver of the non-operated partner. Moreover, in the hepatectomised parabiont the orotic acid incorporation is 30% greater than in the nonparabiotic, hepatectomised rat, indicating an effect of the partner in the promotion of DNA synthesis. Similar effects on the incorporation of orotic acid into the nuclear and cytoplasmic RNA were obtained 36 hours after hepatectomy. The data were interpreted in relation to the total DNA, total RNA, total nitrogen and nuclear counts.

Interrelationship of Plasma Cholate and Phospholipid Concentrations and Their Resultant Effect upon Plasma Cholesterol in Biliary Obstruction

By *Sanford O. Byers and Meyer Friedman.* Harold Brunn Institute for Cardiovascular Research, San Francisco.

After obstruction of the bile duct or massive intravenous injections of bile acid, a marked rise of plasma cholesterol and phospholipid occurs in the rat. The present work is a study of the causal relations between the plasma levels of these three substances: bile acid, phospholipid and cholesterol. In one experimental series rats were infused with bile acid; in a second experimental series the animals were infused with phospholipid. Subsequent analyses of control and infused rats in each series confirmed the effect of bile acid in raising the phospholipid and cholesterol levels. However, elevations of plasma phospholipid, while also leading to an elevation of plasma cholesterol, did not elevate the plasma bile acid. Comparison shows the cholesterol:phospholipid ratios observed in rats at intervals after infusion with phosphatide to be nearly identical with the ratios observed in rats with biliary obstruction. The authors conclude that the elevation of plasma phospholipid, which the excess of bile acid either initially effects or intensifies, is responsible for the hypercholesteremia observed in biliary obstruction.

"Direct" Bilirubin Production in Rat Tissue Homogenates

By *G. M. Grodsky and J. V. Carbone.* Department of Medicine, University of California, School of Medicine, San Francisco.

Recently, several authors demonstrated chemically that "direct" bilirubin isolated from urine, bile and serum is the glucuronide of "indirect" bilirubin. Dutton and Storey, in an earlier report, described a rat liver homogenate system capable of carrying out glucuronide conjugations. We adapted this system to investigate the production, in vitro, of "direct" bilirubin from the "indirect" pigment in normal and abnormal livers.

Incubation of commercial bilirubin (suspended in albumin solution) with liver homogenate resulted in the appearance of "direct" bilirubin as measured by the "1-minute" van den Bergh method. A boiled-water extract containing uridine diphosphoglucuronic acid, previously reported by Dutton and Storey to stimulate glucuronide production in this system, increased the yield of "direct" bilirubin 2- to 3-fold. No synthesis of "direct bilirubin" was observed in the absence of homogenate or bilirubin, or at 0°, or at 0 minutes time. Incubation in the presence of increasing amounts of borneol, reported to be conjugated almost exclusively as the glucuronide in the body, caused a linear inhibition of "direct" bilirubin formation. Incubation with B-glucuronidase caused complete destruction of the synthesized "direct" pigment. When the diazotized "direct" pigment was hydrolyzed and the hydrolysate chromatographed on paper, a spot corresponding to glucuronic acid appeared. A small amount of conjugation activity was observed in the kidney, but none was detected in muscle, brain, spleen, or blood. These results support the reported evidence that "direct" bilirubin is the glucuronide of "indirect" bilirubin.

A comparable synthesis was observed in human liver.

Investigation of liver from rats with congenital hyperbilirubinemia has resulted in the demonstration of a defect in the bilirubin conjugation mechanism of these animals.

INFECTIOUS DISEASES

Type Specific Agglutinins for Group A Hemolytic Streptococci in Human Sera

By *Lowell A. Rantz.* Department of Medicine, Stanford University School of Medicine, San Francisco.

Information regarding the occurrence of type specific antibody production associated with Group A hemolytic streptococcal infection has been difficult to obtain because available methods for study of this phenomenon are cumbersome and unreliable.

Differential centrifugation of young cultures of these organisms has resulted in the production of highly stable suspensions that are rich in M-substance and highly type specific when employed in slide agglutination tests. The reaction is further improved by adsorption of all sera with a single heterologous type which removes nontype specific antibodies.

Specific antibodies capable of agglutinating the homologous type appear in the serum of human beings during recovery from infection and after immunization. Antibodies reacting with various types are present in the serum of healthy adults and in persons suffering from rheumatic fever and naphritis. In the latter instance, type-12 antibodies are nearly always present.

The complexity of the antibody pattern increases with advancing age when healthy children are studied.

The Relationship of Brucella Infection to Cardiovascular Disease in Man

By *Charles M. Carpenter, Donald W. Leik, Joseph A. Schwartz and Richard G. Devereaux.* Department of Infectious Diseases, School of Medicine, University of California, Los Angeles; and the Medical Service, V. A. Hospital, Long Beach.

An epidemiologic study employing intradermal tests for *Brucella* infection revealed a greater prevalence of dermal sensitivity to Brucellergen among hospitalized patients than among a nonhospital population designated as "normal." Further observations revealed that the highest rate, namely, 56%, occurred among a group of male veterans with cardiovascular disease. Among groups of patients with tuberculosis and with gastro-intestinal disease in the same hospital populations, the percentages with dermal sensitivity to Brucellergen were 33% and 29%, respectively.

In order to detect possible cardiovascular disease among patients whose chief illness was other than cardiovascular disease, electrocardiograms were made, following which the occurrence and character of the electrocardiographic changes in *Brucella*-sensitized and nonsensitized patients were compared. Preliminary observations on a group of 36 patients with gastro-enteric disease showed that only 5 of 20 patients with intradermal tests negative to Brucellergen, but 8 of 16 patients with dermal sensitivity to Brucellergen, showed electrocardiographic changes.

Inasmuch as the perivascular tissue is the chief site of the granuloma in experimental brucellosis and this lesion is observed not infrequently in the intima of the arteriole, it is postulated that chronic brucellosis in man may express itself as a cardiovascular syndrome. The significance of the findings cannot as yet be assessed.

The Variability of the Enteric Bacilli in Their Sensitivity to the Bactericidal Activity of Serum from Normal Subjects and Patients with Various Diseases

By *R. J. Roantree and L. A. Rantz.* Department of Medical Microbiology and the Department of Medicine, Stanford University School of Medicine, Stanford.

Normal human serum kills many strains of gram negative enteric bacilli. One ml. of serum may kill over 300 million bacteria of a sensitive strain. The present study was undertaken to determine whether a lack of this lethal effect might play a role in the bacteremias of certain disease states and whether organisms resistant to the effect could be isolated from bacteremic patients.

Serial dilutions of organisms were introduced into 1 ml. portions of human serum which had been kept in a frozen state except for the 2½ hours after drawing. Subcultures were taken after two hours' incubation at 37°. The largest inoculum giving a sterile subculture was designated as the number of organisms killed by 1 ml. of serum. A sensitive strain was designated as one in which over 1 million organisms were killed. A moderately sensitive strain showed the killing of 10,000 to 1 million organisms and a resistant strain showed less than 10,000 killed.

The sera from 14 normal subjects and 52 patients in whom a bactericidal defect might be suspected showed a remarkable uniformity in the ability to kill any given strain. Sera from one patient with lymphocytic leukemia and one with cirrhosis showed a nearly complete lack of the lethal effect.

Strains of bacteria showed marked differences in susceptibility to the effect. Twelve of 14 enteric bacilli isolated from bacteremias were resistant. Two were moderately sensitive, but one of these was recovered from the blood of the patient with cirrhosis whose serum had no bactericidal effect. Investigation of 60 comparable strains isolated from stools and urine showed a decreased incidence of resistance. For example, 5 of 20 strains of *E. coli* were resistant. Of the genera investigated, Shigella and Aerobacter were uniformly sensitive, whereas Salmonella was relatively resistant.

This serum effect depends upon complement and magnesium. Whether properdin described by Pillemer is the only other constituent of the system, or whether naturally acquired antibody plays a role, is not yet certain.

Preliminary Observations on the Use of Nitrogen Mustard in Disseminated Coccidioidomycosis

By *N. B. Kurnick.* V.A. Hospital, Long Beach, and Department of Medicine, University of California, Los Angeles.

Since disseminated coccidioidomycosis is a granulomatous disease associated with hyperplastic reaction in reticulo-endothelial sites, affecting primarily dark-skinned peoples in whom hyperplastic reactivity is common (keloid-formation, lymphogranuloma venerum reactions, etc.), and is associated with complement-fixing antibody production (presumably a reticulo-endothelial product), the titer of which parallels the severity of the disease rather than influencing favorably the course, it appeared of interest to determine the response to a depressant of the reticulo-endothelial system.

Four patients with proven disseminated coccidioidomycosis received one to three courses of nitrogen mustard (HN_2). The clinical effect, response to the coccidoidin skin test, and complement-fixing titer were followed. In each case the skin test became positive or increased in reactivity following therapy and the complement fixation titer fell. The complement-fixing titer continued to fall or remained low for much longer periods than the hematologic depression. The titer tended to rise again after several months, whereupon re-treatment reproduced the original effect.

In two patients, subcutaneous masses and draining skin lesions improved markedly, with complete clinical remission in one. Another patient with widespread disease, including meningitis, showed subsidence of fever, regression of subcutaneous lesions, and improvement in general condition,

but expired following the development of quadriplegia as a result of collapse of an affected cervical vertebra. The course of the fourth patient (a white man) had been benign for some time and was not obviously influenced. He died of a cerebro-vascular accident several months after treatment. A closed osteomyelitic metartarsal lesion revealed viable coccidioides immitis at postmortem. A cerebral coccidioides lesion was apparently inactive.

It appears that HN_2 produces immunologic changes usually associated with clinical improvement, does not in itself destroy the causative organism, and may have a beneficial influence on the course of the disease.

KIDNEY

Renal Regulation of Phosphorus Excretion

By *Ralph Goldman and Samuel H. Bassett.* V.A. Hospitals, Sepulveda and Los Angeles and the Department of Medicine, UCLA Medical Center, Los Angeles. (Aided by grants from the U.S.P.H.S.)

It is generally assumed that the regulation of renal excretion of phosphorus is controlled by the action of parathyroid hormone upon tubular reabsorption of filtered phosphorus. Previous studies performed in this laboratory suggest that variations in the amount of phosphorus filtered may have a greater influence on the amount excreted than is generally realized. In order to test the relative importance of the amount of phosphorus filtered and the amount reabsorbed, six patients were placed on a balance regimen with diets containing 400 mg. of phosphorus daily. After a control period, neutral phosphorus supplements were administered by mouth, then withdrawn, and followed by another control period. Simultaneous creatinine and phosphorus clearances were obtained throughout the entire balance period. It was found that for the first 24 hours increases and decreases in phosphorus load caused changes in serum phosphorus level resulting in alterations in the amount of phosphorus filtered. Adjustments in tubular reabsorption of phosphorus were not seen until after the first 24 hours. There followed an adjustment in tubular reabsorption that eventually accounted for most of the alteration in phosphorus excretion. It appears that alterations in the amount of phosphorus filtered as a result of changes in serum phosphorus level mediate acute changes in urinary phosphorus load. Tubular regulation is important in maintaining the average level of phosphorus excretion, upon which the acute effects are superimposed. If the average level is altered, tubular function assumes a new level. There is no evidence of a significant change in filtration rate. If parathyroid hormone affects tubular reabsorption of phosphorus, the response is a relatively slow one.

Acute Effects of 9αFlurohydrocortisone and 2-Methyl-9αFlurohydrocortisone on Glomerular Filtration and Renal Clearances of Sodium and Potassium in Normal Subjects

By *Jonas H. Sirota, Marcus A. Krupp, Bernard J. Axelrad, G. James Tobias and Jean Fellows.* Palo Alto Medical Research Foundation, Palo Alto, California.

The acute effects of intravenous 9αfluorohydrocortisone (9αFF) and its 2-methyl derivative (2M9αFF) upon GFR and renal clearance of Na and K over a 3½ hour period were determined in 8 normal male adult subjects, employing standard renal clearance technics.

In 3 salt-loaded subjects the control urine Na/K excretion ratios were 5.9, 5.8, and 3.2. Following the intravenous administration of 5.0, 5.0, and 11.7 mg. of 9αFF, respectively, significant changes in these ratios first occurred between 60 and 80 minutes and reached the lowest values of 1.8, 2.2, and 0.7 in 110 to 200 minutes.

In 2 similarly prepared subjects who received 0.5 to 0.7 mg. of 2M9αFF, the urine Na/K ratios changed significantly in 80 minutes with minimum values of 5.0 and 1.9, respectively.

With 9αFF, clearance of K increased to a maximum of 150% over control values in 3 subjects, with significant elevations within 60 minutes. With 2M9αFF, clearance of K did not change in one and, in the other subject, rose to a maximum of 77% over control values.

9αFF produced maximum decreases in clearance of Na of 20% to 50% of control values. Reductions in clearance of Na occurred later than the increases in clearance of K. 2M9αFF produced maximum reductions in clearance of Na of 60% to 70%. This reduction of Na clearance occurred sooner than that following 9αFF.

The administration of both compounds to 3 salt-depleted subjects produced greater reduction in clearance of Na and smaller increases in clearance of K relative to control values than were observed in salt-loaded subjects.

9αFF was administered to 1 patient with adrenal insufficiency. Changes in clearance of K similar to those in salt-loaded subjects were observed. Clearance of Na, however, after an initial fall, rose above control values simultaneously with GFR.

Except for the subject with adrenal insufficiency, both compounds produced insignificant changes in GFR.

The intravenous administration of 9αFF appears to produce a relatively early and marked increase in clearance of K. The decrease in clearance of Na is only moderate and delayed. 2M9αFF appears to produce a smaller effect on clearance of K and a greater and earlier decrease in clearance of Na.

Effects of Standing on Renal Hemodynamics and Water and Electrolyte Excretion in Normal Pregnancy

By *N. S. Assali and P. Titus*. Department of Obstetrics and Gynecology, the University of California at Los Angeles.

It is well known that pregnant woman accumulate a certain degree of edema, which usually subsides at night or following bed rest. The edema is more obvious during the last trimester of pregnancy.

To explain the possible mechanism of this edema, two hypotheses have been advanced: (a) increased hydrostatic pressure in the veins of the lower extremities leading to increased back diffusion pressure; and (b) increased reabsorption of water and electrolytes by the kidneys caused by the high levels of steroid hormones.

To investigate the latter possibility, renal studies were performed on pregnant subjects as early in pregnancy as possible and repeated every 2 months until the time of delivery. Clearances of inulin and PAH were used to estimate GFR and RPF. Three collection periods were obtained in the supine and 3 to 4 in the upright position.

The results showed that upon standing, urine flow decreases from an average of 5 to 0.4 ml./minute with a parallel increase in the U/P ratio of inulin and PAH. Urine osmolarity increased significantly but total solute excretion fell. Similar changes were observed in electrolyte concentrations and excretions. GFR and RPF either remained the same or decreased slightly. All subjects developed postural changes consisting of a moderate hypotension, dizziness, sweating and tendency to fainting. Both the renal and the systemic effects were more marked after the 6th month of gestation.

These data indicate that the primary effect of standing is increased reabsorption of water independently from any change in GFR and RPF. The fall in electrolyte excretion is secondary to increased water reabsorption. Pooling of blood in the lower extremities might be the initial factor that stimulates volume receptor centers leading to increased ADH secretion and increased water reabsorption by the distal tubules.

The Influence of Certain Osmotically Active Substances on the Volume of Dog Kidneys

By *C. A. Nugent and F. H. Tyler*. Department of Medicine, University of Utah College of Medicine, Salt Lake City.

In an investigation of the renal diseases associated with gout, an old experiment of Folin's was repeated. Folin found that the rapid intravenous infusion of lithium urate solutions caused swelling of the kidneys of dogs. After confirming this observation, we found that hypertonic or isotonic solutions of glucose, manitol, inulin and sodium chloride also caused swelling of the dog kidney.

The kidney size was measured by calipers applied to the exposed kidneys of anesthetized dogs. A catheter was placed in the bladder to avoid back pressure on the kidney. The sugars were given as intravenous solutions over 10-minute periods in a dose of 1.5 Gm./Kg. body weight. These infusions resulted in increases in the volumes of the kidneys of 25 to 50%. The blood pressures of the dogs were stable in the experiments. Infusions of concentrated human serum albumin of 1.5 Gm./Kg. gave an immediate increase in blood volume of 27% but did not change the size of the kidneys.

The evidence suggests that a number of osmotically active substances passing down the kidney tubule lumen will cause swelling of the tubule cells.

Biochemical Lesions Observed in Experimentally Induced Renal Tubular Disease in Rats

By *Ruth T. Gross, Carolyn F. Piel, and Harold Harper*. Department of Pediatrics, Stanford University School of Medicine and the Department of Experimental Surgery, the University of California School of Medicine, San Francisco.

The recognition of renal tubular diseases in man has been aided by the application of recent biochemical technics and increased knowledge of renal physiology. Not only have specific clinical syndromes been described but, in recent years, similar derangements have been produced in animals by the use of drugs which act as enzymatic blocking agents. Berliner, in 1950, demonstrated that maleic acid administered to acidotic dogs abolished their capacity to excrete an acid urine and led to increased excretion of endogenous phosphate. Harrison, in 1954, used this drug in rats to produce renal glycosuria, phosphaturia and aminoaciduria, a symptom triad which is found also in a congenital metabolic defect in man known as the de Toni-Fanconi syndrome. The purpose of this study is to present biochemical observations on rats poisoned with maleic acid in an attempt to clarify the action of this drug.

Rats have been poisoned with varying doses of maleic acid in order to confirm Harrison's findings of phosphaturia, glycosuria, aminoaciduria and decreased excretion of citric acid. Observations have also been made on the blood levels of glucose, citric acid and lactic acid and on the levels of citric acid and lactic acid in the tissues. These findings have been compared to similar observations on (1) rats

subjected to starvation and (2) rats poisoned with sodium fluoroacetate.

In rats weighing approximately 250 Gm., maleic acid produced a transient aminoaciduria. Phosphaturia was inconstant. Urinary excretion of citrate increased. Serum lactic acid increased but phosphate, citrate and glucose remained unchanged. There was no significant effect on lactic and citric acid in the tissues. These effects were not reproduced by starvation or administration of sodium fluoroacetate.

The experiment is being repeated on younger rats, and the effects of a rachitogenic diet are being observed.

MUSCLE

Clinical Cardiopulmonary Abnormalities in Dystrophia Myotonica

By *Leonard W. Ritzmann, William W. Thompson, Donald M. Pitcairn and Stanley Welborn.* Department of Medicine, Portland V. A. Hospital, and the Divisions of Neurology and Cardiology, Department of Medicine, University of Oregon Medical School, Portland.

Eight cases of dystrophia myotonica have been studied. All were male patients with ages ranging from 25 to 63 years. The degree of neuromuscular disability varied from mild to severe; none was completely bedridden. The severity of the symptoms was not related to their duration.

Although any cardiac symptomatology was overshadowed by the neuromuscular difficulties, two patients were admitted because of cardiac complaints. One, aged 58, known to have an enlarged heart for two years, entered the hospital because of several episodes of dyspnea, orthopnea, and intermittent ankle edema. ECG showed first degree A-V block and left bundle branch block. The other patient, aged 42, noted increased weakness with the onset of atrial fibrillation. X-ray showed slight cardiac enlargement.

Cardiac abnormalities were found in two other patients. One had second-degree A-V block documented by ECG for 18 years. Another had a bigeminal rhythm from ventricular extrasystoles and later developed short runs or premature atrial contractions. His ECG also showed incomplete right bundle branch block. Cardiac size was normal in these two patients.

Four cases showed no evidence of cardiac disease by examination, ECG, or X-ray.

Impairment of ventilatory function was apparent on inspection and fluoroscopy in the most severe cases and mild secondary polycythemia was found in four cases.

Due to the sparsity of more detailed reports of the cardiac and pulmonary physiology in this disease, hemodynamic investigations with cardiac catheterization and pulmonary function studies are being carried out in all of these patients.

Cardiopulmonary Function in Patients with Dystrophia Myotonica

By *Herbert E. Griswold, Jr., Donald M. Pitcairn, Stanley Welborn, Leonard W. Ritzmann and William W. Thompson.* Divisions of Cardiology and Neurology, Department of Medicine, University of Oregon Medical School, and the Department of Medicine, V. A. Hospital, Portland.

The clinical manifestations of cardiopulmonary disability in dystrophia myotonica have been previously described. Cardiopulmonary function in three patients with this congenital syndrome has been studied by means of venous cardiac catheterization and the simpler tests of ventilatory function. Cardiac output was determined according to the direct Fick principle by simultaneous collection of inspired air and sampling of mixed venous blood and systemic arterial blood. Pressure pulse contours were obtained from the right side of the heart and from peripheral systemic arteries.

The patients were all males aged 42, 59 and 63 years. The range of the cardiac index was from 1.28 to 2.04 liters per minute per M^2 body surface area. In two patients the oxygen consumption was normal and the low cardiac output was therefore a reflection of an increased A-V O_2 difference. Pulmonary artery and ventricular systolic pressures were within the normal range. In one patient, however, there was a marked elevation of right ventricular and diastolic pressure and right atrial mean pressure. End diastolic right ventricular pressure in this patient was 16 mm. Hg. Systemic arterial pressures were all within normal range. Systemic peripheral resistance was somewhat increased in all subjects.

Ventilatory function was assessed by measuring the timed vital capacity and maximum breathing capacity. (Collins Respirometer.) In all patients the vital capacity was significantly reduced below the normal value predicted from the regression equation of Cournand et al. The expiratory dynamics were normal in all subjects, as reflected in the one- and two-second portions of the vital capacity. The maximum breathing capacity was reduced in all subjects.

The Uptake of Radioiron into Myoglobin in Cell-Free Extracts of Dog Muscle

By *Gerald T. Perkoff and Frank H. Tyler.* Department of Medicine, University of Utah College of Medicine, Salt Lake City. (Aided by grants from the Muscular Dystrophy Associations of America and the U.S.P.H.S.)

Studies of the synthesis and degradation of myoglobin, of great interest in the understanding of diseases of muscle, have been hampered by the lack of a simple method for the isolation of hemoglobin-free myoglobin. By the use of a method based on the relative solubility of these proteins in concentrated phosphate buffers, it has been possible to isolate myoglobin free of hemoglobin and to study the incorporation of Fe^{59} into myoglobin in cell-free buffered extracts of dog muscle.

Dog muscle was collected over ice, ground, homogenized in Krebs-Ringer phosphate buffer, and centrifuged. The supernate was passed through a Seitz filter. After the addition of radioiron, multiple samples of extract were incubated with shaking at 37° for periods up to 72 hours. Myoglobin was then isolated from each sample and the radioactivity determined in a well-type scintillation counter.

Radioiron incorporation into myoglobin reaches an initial peak in one to eight hours and then returns to lower levels in 24 hours. After this time, a progressive rise in myoglobin radioactivity occurs, reaching a second and greater peak in the subsequent 48 hours. Incubation mixtures containing saponin, heated to 56° for 30 minutes prior to the addition of iron, or carried out under nitrogen, show only the late rise in myoglobin radioactivity. The addition of KCN results in augmentation of the initial peak, with partial depression of the secondary rise.

Tiselius electrophoresis of myoglobin prepared in the manner described demonstrates a major and a minor component. The relationship of the double peak of incorporation of radioiron into myoglobin to the two components of myoglobin demonstrated by electrophoresis is under study.

NEOPLASTIC DISEASE

Influence of Graded Psychologic Trauma on Tumor Development in the Rat

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In an objective evaluation of the effects of graded psychologic trauma upon the development of experimental tumors in the rat, varying permutations of tumor induction and types of stress were studied. Three types of tumors were used: sarcomas induced by methylcholanthrene, the transplanted Walker carcino-sarcoma and Murphy-Sturm lymphosarcoma. Wistar rats were subjected to stresses of varying duration involving continued inevitable punishment by electric shock with prolonged anticipatory signals before the onset of the stimulation. Both sexes and different age levels were studied. Also included were the effects of prior and/or concurrent stress with tumor induction or transplantation. Ten different groups of rats were evaluated. For the most part, results appeared to be inconclusive insofar as definite trends of tumor development were concerned. An exception to this was that tumor growth appeared to be affected more by the past stress treatment of the rat than stress following tumor induction or transplantation. Thus, of 59 rats distinguished only by the fact that 30 had been stressed for a 50-day period prior to transplantation with the Murphy-Sturm tumor and 29 had received no stress, the group with stress background developed 15 tumors, as compared to 8 in the control unstressed group. Following the transplant no stress had been given to either group. Final statistical evaluation of these studies is not yet completed. Additional aspects of this program include studies of the influence of adrenalectomy and stereotaxis destruction of the median eminence of the hypothalamus upon tumor development. Such intervention as this, when accompanied by stress, appears to have more significant influence on the tumors than simple stress alone. Results such as these would seem to suggest a more complex mediating relationship between stress and tumor development than would be implied by gross clinical interpretations.

Clinical Evaluation of Chlorambucil in the Treatment of Human Neoplastic Disease

By *B. E. Hall, F. M. Willett, T. V. Feichtmeir, D. R. Hales, R. W. Jerner and J. Franco.* Department of Medicine, Stanford University School of Medicine, and San Francisco V. A. Hospital. (Aided by a grant from the National Institutes of Health.)

Clinical trials with the aromatic nitrogen mustard, p-(di-2-chloroethylamino)-phenylbutyric acid (C.B. 1348, Chlorambucil, or Leukeran) have been carried out in 95 patients having various types of neoplastic disease during the past 2½ years. This series includes 46 patients with various types of malignant lymphoma, 9 patients with chronic

lymphocytic leukemia, 1 patient with subacute lymphocytic leukemia, 2 with myeloma, and 33 with metastatic solid tumors. The oral route of administration was utilized in all cases. Dosage schedules varied from 0.5 to 0.4 mg./Kg. of body weight/day, the compound being given continuously over periods lasting from 2 or 3 weeks to several months, and in certain instances, intermittently for periods of from 18 to 20 months.

Beneficial results were observed in a significant number of patients having Hodgkin's disease, lymphosarcoma, giant follicular lymphoma, and chronic lymphocytic leukemia. One patient with far advanced Hodgkin's sarcoma has been maintained in a reasonable state of health for a period approaching 2 years. Occasional cases with metastasis due to undifferentiated carcinoma respond well also, as manifested by an individual with extensive pulmonary metastasis who was strikingly benefited over a period of 16 months. The compound was ineffective in reticulum cell sarcoma, in myeloma, and in well-differentiated solid tumors.

The principal toxic manifestation was suppression of hemopoiesis, characterized by leukopenia, thrombocytopenia, and/or anemia, that developed after several weeks or months of continuous therapy. Recovery usually was prompt following cessation of treatment. However, one death probably attributable to the drug was observed.

Effect of Protein Feeding on Serum Enzyme Levels in Patients with Metastatic Carcinoma

By *Laurens P. White.* Stanford University School of Medicine, San Francisco.

About 40% of patients with widespread malignant tumors can be shown to have elevated levels of several glycolytic enzymes in their peripheral blood. The reasons for the abnormality are obscure, and abnormal serum levels of these enzymes are found in many other illnesses, most notably in progressive muscular dystrophy, hepatitis, and after myocardial infarction.

Using the general method of differential spectrophotometry, serum levels of aldolase, hexose isomerase, lactic dehydrogenase and glutamic oxalacetic transaminase were measured in more than 250 patients. From this group, those with abnormal serum levels of the enzymes were taken for study. Serial enzyme measurements and urinary creatine-creatinine estimations were performed during a control period while patients were receiving a diet of known caloric and protein composition. The protein intake was then increased, either by oral supplementation or intravenous administration of amigen or albumin. The various measurements were continued during this period.

In all but one of the patients with metastatic cancer so studied, the levels of the various enzymes in the peripheral blood fell to normal or almost normal with adequate protein alimentation. Creatine levels in the urine regularly decreased. In suitable control subjects such changes were not seen after protein hyper-alimentation.

It is concluded that in most patients with widespread cancer, abnormal serum levels of glycolytic enzymes are a reflection of muscle wasting secondary to relative starvation of the host. This muscle wasting is directly related to two factors: (1) Tumor acts as a nitrogen "trap" and pre-empts the largest share of protein precursors from the diet and from the amino acid pool of the body. (2) Under such circumstances ordinary (or, as frequently happens, reduced) amounts of dietary protein are insufficient to meet the protein requirements of both tumor and host.

The muscle wasting and abnormal serum enzyme concentrations can be reversed by adequate protein intake in patients with cancer.

RESPIRATORY SYSTEM

Ideal Lung Ventilation in Women and Children

By *Rex L. Huff and Daniel Parrish.* Radioisotope Service, V. A. Hospital and the Department of Medicine, University of Washington, Seattle.

The ventilation patterns of children and women, studied with hydrogen-3 (H^3) on an open system, suggest in most cases that each breath is perfectly mixed in the entire functional residual capacity. The log of the concentration of H^3 in the washout phase, when plotted against time, is a straight line. Twelve children (16 measurements) of both sexes have been studied, all of whom show a single component mixing characteristic. Nine of 13 women have qualitatively similar data. This is in contrast to the data of male adults, which always show at least two time constants for the washout of H^3. With the occurrence of emphysema there is frequently more than two components. This finding of "ideal air mixing" in children and women is thought to be more than fortuitously related to their very low incidence of emphysema. The data suggest an inherent structural and/or functional difference in the lungs of males to account for their relatively high incidence of emphysema. Many authors have suggested that this difference between sexes is on the basis of environment or occupation. Insufficient data have been accumulated to enable us to determine the age when "ideal mixing" of boys changes to the typical male, adult, complex, type. "Ideal mixing" has been found only rarely with the nitrogen technic and never with helium. The slow response time of the helium katharometer modified the natural phenomenon, while the continued release of nitrogen from the blood prevents accurate interpretation of the late parts of the nitrogen "washout curve."

Studies on the Alveolar-Arterial Oxygen Gradient in Acute and Chronic Poliomyelitis

By *Stanley N. Rokaw, John E. Affeldt, Clarence R. Collier, Milton G. Crane and Andrew F. Farr.* Respiratory Center for Poliomyelitis, Rancho Los Amigos Hospital, Hondo, California.

Previous observations have demonstrated the existence of an increased alveolar-arterial oxygen gradient in some poliomyelitic respirator patients. Evaluation of the relative importance of diffusion and distribution components of the gradient, in both acute and chronic phases, was undertaken.

Pulmonary gas exchange has been studied in both patients and normal individuals, employing the two-level oxygen breathing technic and tension determination methods of Riley, et al. Simultaneous collections of total expired gas and arterial blood, during measured intervals, were made. Twenty-nine studies in 26 chronic patients, 10 studies in acute patients, and 4 in three normal individuals were completed satisfactorily.

Results (mean values) calculated for the following parameters, for polio and normal groups respectively, follows: Alveolar-arterial oxygen gradient (mm. Hg) (breathing air), 22.4, 15.8%; dead space/tidal volume-ratio (ventilatory shunt), 33, 21%; venous admixture/total pulmonary blood flow-ratio (circulatory shunt), 10.5, 6.7%; diffusing capacity for oxygen (resting) (ml./min./mm.Hg/M^2), 8.2, 9.4%.

Data accumulated indicate some moderate abnormality in pulmonary gas exchange in the respirator patients, largely related to disturbances in distribution of blood and gas in the lungs. Results for some patients, however, fall within the range of values of the normal population. Variations are not as marked as observed in other diseases where structural change in the lung can be identified. The role of recumbency, fixed tidal volumes, or other mechanical characteristics of positive pressure respiration in these patients, in producing these changes, is suggested by this and by corollary experimental work.

RHEUMATIC STATES

Acute Arthritic Lesions in the Guinea Pig Induced with C^{14}-labeled Bacterial Polysaccharides

By *Russell S. Jones.* Department of Pathology, University of Utah College of Medicine, Salt Lake City.

Acute joint lesions have been induced in the guinea pig by polysaccharides and somatic antigens of certain gram-negative bacilli. Joint exudate and synovial proliferation occurs within hours after a single intravenous injection of bacterial polysaccharide. The toxicity of the bacterial products is associated with precipitation of bile in the gall bladder, the appearance of protein-rich ascitic fluid and PAS-positive, diastase-digestible masses in the hepatic parenchymal cells. These evidences of toxicity, however, are not necessary for the production of joint lesions; a nontoxic, nonantigenic hapten induces the acute arthritic lesions.

Since the tissue distribution and metabolism of the polysaccharide complexes from bacteria would aid in understanding the pathogenesis of the joint lesions, K. pneumoniae organisms have been labelled with C^{14} by biosynthesis and the polysaccharide-complexes extracted by several technics. Tissue distribution was followed by determining isotopic activity in tissue "homogenates" and by autoradiography after a single intravenous injection. Respiratory $C^{14}O_2$ loss is exponential for the first 2 days. Urinary C^{14} loss rapidly declines to a fairly stable level for several weeks. Tissue localization is predominantly within reticuloendothelium. Maximum C^{14} activity in most organs and tissues is rapidly attained, then declines, disappearing within two months. Surprisingly, the adrenal cortex has the highest C^{14} concentration, per gram, of any tissue; in contrast to other tissues, the adrenal C^{14} uptake reaches its maximum at 4 to 7 days. Little of the C^{14} is in the adrenal lipid.

Considerable C^{14} is present in the fluid aspirated from the joints and autoradiographs showing a dense concentration in the synovium. While this suggests that the joint lesions may result from the direct action of foreign mucopolysaccharides within the synovium, an "indirect" effect is suggested by the injection of plasma from a toxic animal into a normal animal. Although little bacterial polysaccharide is present in the transfused plasma, lesions appear more rapidly in the plasma-injected than the original polysaccharide-injected animal.

SKIN

The Nature of the Tissue Damage Produced by Experimental Frostbite

By *Frederick A. Fuhrman and Geraldine J. Fuhrman.* Department of Physiology, Stanford University School of Medicine, Stanford.

Tissue damage produced by frostbite (freezing) has been attributed both to a direct effect of freezing on the tissues and to secondary vascular changes following thawing. We have investigated (a) tissue loss following standard frostbites in rats, (b) respiration and glycolysis of skin removed from frost-

bitten feet of rats and (c) respiration and glycolysis of tissues after freezing and thawing in vitro. The extent of tissue loss and impairment of metabolism produced by freezing in situ or in vitro are dependent upon the temperature during the frozen state and its duration. The metabolism of skin is more resistant to freezing than that of other tissues (muscle, nerve). The respiration of skin removed from frostbitten feet immediately after thawing is reduced quantitatively the same as that of skin frozen in vitro when the conditions of freezing and thawing are comparable. After brief frostbite (−25°C. for 1 min.) the respiration of skin removed from frostbitten feet at various intervals (up to 2 days) after thawing was further reduced as the time after thawing increased. The data are interpreted to mean that part of the damage following brief frostbite is the direct result of freezing per se and part is the secondary result of changes following thawing. Comparison with results obtained by freezing skin in vitro suggests that with longer frostbite (about 3 hrs. at −25°C.) the damage to skin is maximum and complete at the time of thawing. Therapy by immediate rapid warming, which is remarkably successful following brief frostbite, was unsuccessful following frostbite produced by immersion for 3 hours at −20°C.

Competitive Inhibition of Mammalian Tyrosinase by Phenylalanine and Its Relationship to Hair Pigmentation in Phenylketonuria

By *Masamitsu Miyamoto and Thomas B. Fitzpatrick*. University of Oregon School of Medicine, Portland.

In this study an attempt is made to establish a relationship between high blood levels of phenylalanine and its derivatives, and hypopigmentation of skin and hair in phenylketonuria. As a source of mammalian tyrosinase, dialyzed and lyophylized melanin granules from Harding-Passey mouse melanoma were used. Tyrosinase activity was determined manometrically in the Warburg respirometer at 38°C. with 0.1 M sodium phosphate buffer at pH 6.8. Each Warburg vessel contained 1.4–1.5 units of tyrosinase.

L-phenylalanine was found to be an inhibitor of mammalian tyrosinase ($-\text{Log } I_{50} = 2.0$). Using Lineweaver and Burk's method of plotting, L-phenylalanine was shown to be a competitive inhibitor of tyrosine-tyrosinase activity. The other abnormal metabolites which are reported to occur in the blood or urine of phenylketonurics were studied in the same manner. Phenylpyruvic acid (sodium salt) and phenylacetic acid showed weak inhibitory effect, whereas p-hydroxyphenylacetic acid had the most marked inhibitory effect ($-\text{Log } I_{50} = 2.8$). D-phenylalanine and DL-alanine failed to show any detectable inhibitory effect even at relatively high concentrations of 8.7 and 8.2×10^{-3} M, respectively. Inhibition of mammalian tyrosinase by L-phenylalanine at the same concentration as in the blood serum of the patient is approximately 15–30% under the experimental conditions used. However, both enzyme (tyrosinase) and substrate (tyrosine) are present in the human skin and hair follicle in much smaller amounts and greater inhibition by L-phenylalanine can be expected in vivo.

The other metabolites in the serum of phenylketonurics are present in too low a concentration to result in any significant inhibition in vivo. On the basis of this study, it is believed that the hypopigmentation noted in phenylketonuria may be related to the inhibition of melanin formation by the abnormally high serum levels of L-phenylalanine. It is also possible that L-phenylalanine may be one of the regulating factors in the rate of melanin synthesis in normal skin and hair.

THERAPEUTICS

Anti-Inflammatory Properties of a Pyrimidine Derivative, RO 2-5383/2

By *Cutting B. Favour*. Department of Immunology, Palo Alto Medical Research Foundation, Palo Alto. (Aided by a grant from the Roche Memorial Foundation.)

A new anti-inflammatory compound, Ro 2-5383/2, has recently been reported to have approximately half the antiphilogistic effects of cortisone. The mechanisms of action of this potentially useful drug have been explored in the present study.

Similar local inflammatory skin lesions were produced in guinea pigs by (1) a mild non-specific irritant (autoclaved yeast particles); (2) a toxic, non-specific irritant (turpentine); and (3) specific delayed-type allergy (tuberculin reactions). Animals treated with 25 mg.%/Kg. body weight, twice daily showed significant suppression of all three types of inflammation. Control animals treated with cortisone-hemisuccinate, 10 mg.%/Kg. body weight intramuscularly, twice daily, showed a greater suppression of reactions. Local injection of either drug with test materials gave similar results. White blood cell counts, blood sugar determinations and urinary 17-ketosteroid excretion studies indicate that Ro 2-5383/2 does not act via alteration in the blood count or activation of adrenal function. Control studies with hydrocortisone exhibited the expected changes in leucocyte counts, blood sugar values, and steroid excretion. It is concluded that this drug is useful for studying the pathogenesis of inflammation. It is one of a new family of compounds with potential clinical usefulness in the treatment of inflammatory and allergic disorders.

PROGRAM, SOUTHERN SECTION

American Federation for Clinical Research

Friday, January 25, 1957

Jung Hotel, New Orleans, Louisiana

Dr. Samuel P. Martin, Presiding

9:00 a.m.

1. **Type-specific Antibody Response to M Protein of Nephritogenic Streptococci in Glomerulonephritis.**
   Mary Alice Bone, A. I. Braude and Herman Kleinman,** Dallas.
   page 115
2. **Two Red Cell Populations During Mild Crisis in Sickle Cell Anemia.**
   John H. Moon and G. Watson James, III,* Richmond.
   page 90
3. **Megaloblastic Anemia Associated with Excretion of Orotic Acid: A New Entity.**
   Charles M. Huguley, Jr., James A. Bain, Shirley L. Rivers* and Robert Scoggins,** Atlanta.
   page 90
4. **Studies on Circulating Erythropoietin in Anemic Human Subjects.**
   A. Leonard Luhby, Sam J. Piliero, Paul T. Medici,* Ben Pansky* and Albert S. Gordon,** New York.
   page 89

INTERMISSION

5. **Microelectrophoretic Studies of Human Blood Platelets.**
   *S. William Ross,** Little Rock, Arkansas. (Introduced by *E. J. Towbin.*)
   page 93
6. **Studies on Testicular Interstitial Cell and Adrenal Cortical Response to Adrenocorticotropin and Human Chorionic Gonadotropin in Man.**
   Lester L. Hibbett, Willard R. Starnes* and S. Richardson Hill, Jr.,* Birmingham.
   page 106
7. **On the Mechanism of the "Shunt" Pathway of Uric Acid Synthesis.**
   James B. Wyngaarden, Alberta Blair and Janet Schuck,** Bethesda.
   page 108
8. **Excretion of Intravenously Administered Creatine by Healthy and Myopathic Humans.**
   *Nikos G. Bourdakos,** Oklahoma City. (Introduced by *Stewart Wolf.*)
   page 117

* By invitation
† Senior Member

9. **Studies of the Satiety Response in Mice.**
   Guy Hollifield and William Parson,† Charlottesville, Virginia.
   page 104
10. **A New Tool in the Diagnostic Evaluation of Cushing's Syndrome.**
    Grant W. Liddle, William S. Coppage, R. Glenn Greene* and Donald Island,** Nashville.
    page 106
11. **Adrenocortical Function in Myxedema.**
    Gerald A. Williams, K. R. Crispell and William Parson,†* Charlottesville.
    page 104

2:00 p.m.

BUSINESS MEETING

Chairman's Address

12. **A Thyroid-Stimulating Hormone Assay Based on I^{131} on Rana Catesbeiana Tadpoles.**
    C. Y. Bowers and Albert Segaloff,* New Orleans.
    page 109
13. **The Effect of Unsaturated Fats on Serum Lipids in Normal, Active Subjects.**
    William Shapiro, E. Harvey Estes, Jr. and Helen L. Hilderman,** Durham.
    page 107
14. **Effect of DOCA Upon the Development of Reno-Prival Hypertension.**
    H. G. Langford, J. R. Snavely and Don M. Turner,** Jackson.
    page 98
15. **Relation of Serum Bicarbonate to Bicarbonate Diuresis During Diamox Administration.**
    Richard M. Portwood, Floyd C. Rector, Jr., Robert Cade* and Donald W. Seldin,* Dallas.
    page 113
16. **The Effect of Dilution and Dehydration in Patients with Edema and Hyponatremia.**
    Mackenzie Walser and Jack Orloff, Bethesda.
    page 114

INTERMISSION

17. **The Influence of Pulmonary Capillary Blood Flow upon Alveolo-Capillary Gas Exchange.**
    Peter C. Luchsinger, Georges F. McCormick and Kenneth M. Moser,** Washington, D. C.
    page 118

Advance Reports Submitted to the Annual Meeting of the Southern Section of the American Federation for Clinical Research

Jung Hotel, New Orleans, Louisiana · Friday, January 25, 1957

BLOOD

Studies on Circulating Erythropoietin in Anemic Human Subjects

By *A. Leonard Luhby, Sam J. Piliero, Paul T. Medici, Ben Pansky and Albert S. Gordon.* New York Medical College, Flower-Fifth Avenue Hospitals, St. John's University and New York University, New York City.

Studies were undertaken to detect the presence of a circulating erythropoietic stimulating factor in the plasma of human subjects with severe anemia. It has now been well documented that laboratory animals subjected to anoxia develop a circulating plasma factor capable of stimulating increased erythropoiesis in the normal animal. A variety of reports have suggested that such a factor in human beings may mediate the secondary polycythemia associated with living at high altitudes or with pathologic pulmonary and cardiac conditions resulting in chronic anoxia. Few studies, however, have attempted directly to assess the erythropoietic stimulating qualities of certain body fluids of human subjects.

Materials and methods: The plasma of patients with Cooley's anemia, sickle cell anemia and chronic hypoplastic anemia, as well as the urine of patients with Cooley's anemia, were evaluated for "erythropoietin" as outlined below. The plasma and urine of normal subjects of the same age group were employed as controls.

The assay procedure employed involves the preparation of a "protein-free" extract of plasma and urine. The material, brought to a pH of 5.5, is boiled for ten minutes. It is then filtered, the solids discarded, the filtrate brought to a pH of 7.2, adjusted with saline to the original volume of plasma and injected into a modified Long-Evans strain of rats. Each of 2 to 5 rats received a subcutaneous injection of 1.8–3.0 ml. daily for five days. Urine samples were treated in the same manner except that the final material was at half the volume of the original urine. Peripheral blood studies were done on all the animals prior to, and at the end of, the sixth day study period. Bone marrow examinations were done after sacrifice on the sixth day.

Results: Striking increases of the circulating red blood cell count, hemoglobin, hematocrit and reticulocyte count, as well as the bone marrow nucleated red cell count of the rat, were obtained with 6 of 7 plasmas from patients with Cooley's anemia and with 1 of 2 sickle cell anemia plasmas. The plasma of a patient with chronic hypoplastic anemia was inactive. The urine of two patients with Cooley's anemia whose plasma showed activity also possessed erythropoietic stimulating activity.

It is apparent from these studies that the plasma and urine of patients with Cooley's anemia and the plasma of certain individuals with sickle cell anemia possess a circulating erythropoietic stimulating factor. It is of interest that a patient with a chronic anemia associated with an absence of erythrocyte precursors in the bone marrow had no such circulating "erythropoietin."

Studies of Plasma Erythropoietic Factor in Human Anemia

By *T. C. Prentice and E. A. Mirand.* Roswell Park Institute, Buffalo, New York.

During the past several years, increasing evidence has been accumulating to show that a humoral factor may be important in regulating red cell production. More recently, it has been shown that a "deproteinized" plasma extract from anemic animals is capable of stimulating erythropoiesis in recipient animals. Cross-species assay thus becomes possible.

The present study has been carried out (1) to develop a simple test for erythropoietic factor in "deproteinized" plasma that is applicable to the study of anemia in humans; and (2) to apply this to the various types of anemia in humans and to correlate the type of anemia with the quantity of erythropoietic factor present in the plasma.

Such a test has been devised, utilizing three times concentrated deproteinized plasma (anemic and control) and injecting this material subcutaneously for three days into hypophysectomized rats. Fe^{59} uptake into red cells and reticulocyte counts are the criteria of stimulation. The present method enables a rapid test of plasma from an anemic source, requires only small amounts of plasma and yields clear-cut differences (3- to 6-fold) between anemic experimental and control animals.

Applying this test to human subjects, we have studied 15 patients to date. Ten of the 15 patients have had significant amounts of EPF in their plasma. (Experimental animals incorporated 2 to 3 times more Fe^{59} into RBC than controls receiving normal deproteinized plasma.) At the present time, more detailed studies of the recipient rats (quantitative and qualitative bone marrow analysis) and the donor humans (bone marrow and blood morphology, combined Fe^{59}-Cr^{51} studies to assess production and destruction) are being carried out.

Megaloblastic Anemia Associated with Excretion of Orotic Acid: A New Entity

By *Charles M. Huguley, Jr., James A. Bain, Shirley L. Rivers and Robert Scoggins.* Emory University School of Medicine and the Emory University Hospital, Emory University, Georgia.

Orotic acid, a pyrimidine considered to be a precursor of uracil, to which it is converted by decarboxylation, has been reported to be effective in pernicious anemia. Our attention was attracted to this metabolite when it was identified as the material responsible for massive crystalluria in a child with refractory megaloblastic anemia. Since then we have searched for orotic acid by Dowex column chromatography in the urine of 39 people. None was found in the urine of 15 nonanemic children aged 3 months to twelve years, 10 patients with pernicious anemia or nutritional megaloblastic anemia, or 12 patients with various conditions. Small amounts of a material provisionally identified as orotic acid were found on one occasion in the urine of a child with pure red cell aplasia of the marrow (congenital hypoplastic anemia), and in the urine of an adult with a refractory anemia. The original patient, however, has excreted very large amounts, as much as 1,000 mg. in 24 hours.

M.R., a 28-month-old white male infant, was first seen in March, 1955, with a severe microcytic hypochromic anemia which had not responded to iron. He was noted to have blue sclerae but no other obvious congenital defect. A bone marrow was strikingly megaloblastic. There was massive crystalluria which, on three separate occasions, has caused urethral obstruction. Studies revealed these crystals to be orotic acid. The patient did not respond to Vitamin B_{12}, folic acid, ascorbic acid, or to pyridoxine. The Schilling test revealed a normal absorption of Vitamin B_{12}. He was placed on adrenal steroids, with remarkable increase in hemoglobin, although the hypochromic anemia was not completely relieved. The bone marrow did not revert entirely to normal, but megaloblastic changes were much less striking. On two separate occasions, steroids were gradually withdrawn and each time the patient suffered a relapse of his anemia. Studies on the patient's family have been negative for any similar disorder in parents or siblings.

Two Red Cell Populations During Mild Crisis in Sickle Cell Anemia

By *John H. Moon and G. Watson James, III.* Laboratory for Clinical Investigation, Department of Medicine, Medical College of Virginia, Richmond.

Other investigators using either the Ashby or Cr^{51} methods for erythrocyte survival have suggested that the disappearance rates of erythrocytes in some patients with sickle cell anemia indicate two populations of red cells with different survival times. The present study was undertaken to reinvestigate these observations using *simultaneous* Cr^{51}- and N^{15}-labeling technics.

A 35-year-old Negro female with proven homozygous sickle cell disease (95% S—5% F) has had relative freedom from serious clinical crises and was in a stable hematologic state with a hemoglobin of 7.2 Gm.%, RBC 2.1 million, and reticulocytes of 30%. She was fed 500 mg. of N^{15}-glycine (62 atom % N^{15}) and simultaneously was given an autotrans-

fusion of 20 cc. of whole blood tagged with approximately 70 microcuries of Cr^{51}. For the next week she had symptoms of mild crisis with a fall in hemoglobin to 5.9 Gm. %. The rate of appearance and disappearance of the N^{15} label was followed in her blood as hemin and in her feces as stercobilin for 50 days. The rate of Cr^{51} removal from the blood was followed over the same period of time.

Analysis of the curves obtained suggested two populations of red cells. The uncorrected Cr^{51} data is interpreted as showing a T½ (half-life) of 4 days and a T½ of 24 days (normal 33 to ±3 days). The newly formed N^{15}-labeled hemin data indicated a population with random destruction (T½ of about 6 days), and a second population without random destruction approximating a normal life span. Stercobilin excretion data are difficult to interpret since the curve incorporates three different components.

By two different technics performed simultaneously, a normal and a short-lived population of both preformed and newly-formed erythrocytes have been demonstrated in a patient with sickle cell anemia during a mild crisis.

A New Syndrome: Hemoglobin SC Thalassemia, with Additional Observations on Hemoglobin C Thalassemia

By *Charles L. Brown, Jr., Charles C. Sprague and Henry W. Kloepfer*. Department of Medicine, Tulane University School of Medicine, and the Medical Service, V.A. Hospital, New Orleans.

In recent years, the study of human hemoglobin has led to the definition of several new clinical syndromes. It has been predicted that additional entities will be described. We should like to report on a Negro family in which the father had C thalassemia and the mother had sickle cell trait. Their 11 children were found to have various combinations of these anomalies. Two were believed to represent the previously undescribed entity hemoglobin SC-thalassemia.

The father had a mild microcytic normochromic anemia, numerous target cells, and splenomegaly. Electrophoresis of his hemoglobin revealed 78% C and 22% A. Fetal hemoglobin was normal by the alkaline denaturation method. Erythrocyte survival studies indicated a shortened life span. With these findings it was concluded the father had the hemoglobin C trait plus the thalassemia trait. His sister had almost identical findings on electrophoresis and was thought to have the same abnormality. The mother showed uncomplicated sickle cell trait.

The propositus and two siblings had SC hemoglobin. The propositus was first seen during an episode of bacterial pneumonia and had a mild hemolytic crisis with splenomegaly. Subsequent observations revealed normocytic, normochromic erythrocytes with anemia, numerous target cells, moderately decreased osmotic fragility and shortened erythrocyte survival. The spleen was not palpable. He had 55% S and 45% C hemoglobin and was considered to represent the sickle cell-hemoglobin C syndrome. The other two siblings, with SC hemoglobin, differed in several respects. Neither had ever been seriously ill. The spleen was palpable in both. They were mildly anemic with strikingly microcytic but normochromic red cells. Target cells were numerous. There was marked resistance of the erythrocytes to hypotonic salt solutions. Electrophoresis revealed a greater proportion of C than S hemoglobin. There was no increase in fetal hemoglobin. Red cell survival studies indicated mild hemolysis.

The striking difference between these two patients and their brother with SC hemoglobin, plus findings indicating thalassemia trait in other members of the family, led us to believe they represented the first examples of the anticipated SC thalassemia syndrome.

The Relationship of $Cobalt^{60}$ Vitamin B_{12} Urinary and Fecal Excretion and Liver Uptake

By *P. C. Johnson, W. L. Scaff, D. L. Patrick and E. S. Berger*. Radioisotope Service, V.A. Hospital, Oklahoma City.

$Cobalt^{60}$ Vitamin B_{12} is a convenient way to measure Vitamin B_{12} absorption. This may be done by measuring either fecal or urinary excretion or liver uptake of the labeled vitamin. These technics adequately differentiate lack of intrinsic factor from other pathologic condition. The addition of the intrinsic factor to the tracer dose enhances B_{12} absorption but does not bring these tests consistently into the normal range. These technics have been used extensively in pernicious anemia research. In almost every case, this research is performed and reported using only one of the three methods of measuring B_{12} excretion.

We have analyzed Vitamin B_{12} absorption tests using standard technics for urinary and fecal excretion and using 4″ × 3″ plastic phosphor for liver uptake. The values obtained in our hands were: (Mean ± 1.96 S.D.); Normals, 5.8–24% excreted in urine, 5.1–33% in stool; pernicious anemia, 0.2–6.8% excreted in urine, 45–100% in stool. Two of these technics were performed in each patient simultaneously.

Our data show very poor mathematical correla-

tion (correlation coefficient: $p > .05$) between any two of these radioactive technics in both patients without known neurologic or hematologic disease analyzed in groups of 10 patients and in 8 patients with pernicious anemia. This has occurred in spite of the fact that liver counting is sensitive enough to differentiate patients given a loading dose from those not given a loading dose ($p < .001$).

Each of these tests is measuring a different aspect of the metabolism of Vitamin B_{12} and it suggests that they are necessarily related when measured by these technics.

Our previous work has shown that the site of absorption and/or contact with intrinsic factor may affect urinary excretion in a different way than it does fecal excretion. This and other factors may be related to the failure of liver uptake and urinary excretion to correlate with each other.

These findings show that further research is necessary in order to elucidate the physiologic meaning of these tests; until this has been accomplished, further research into the absorption and excretion of labeled Vitamin B_{12}, with and without intrinsic factor, should include all three of these tests.

Renal Hemodynamics in Polycythemia Vera

By *H. V. Murdaugh, Jr.* Department of Medicine, Duke Hospital, Durham, North Carolina.

Previous workers studying the renal hemodynamics in polycythemia vera have reported abnormal clearances with reduced glomerular filtration rate and renal plasma flow. Since a number of the patients in these studies had coexistent renal disease, it does not appear established that the reported abnormal clearances were manifestations of the elevated hematocrit alone. Accordingly, patients with polycythemia vera were screened for study. Young patients with no evidence of renal disease by urinalysis and urine concentrating ability, without hypertension, and with hematocrits of over 60% were sought. In a 28-month period, only three patients were found who fulfilled these requirements.

These patients were studied using standard renal clearance technics before and after the hematocrit was lowered by phlebotomy. It was found that the effective renal blood flow and the glomerular filtration rate were normal before phlebotomy and did not change beyond the limit of technical error after phlebotomy. The effective renal plasma flow, however, was diminished before phlebotomy and increased to normal after phlebotomy. These patients maintained a normal rate of glomerular filtration by increasing the fraction of plasma that was filtered. Renal vein catheterization was successful before phlebotomy in two of the patients with normal sodium para-aminohippurate extraction (86% and 91%).

The findings in these patients agree with the findings reported in secondary polycythemia (Scott and Elliott), and in experimental polycythemia (Spencer), and suggest that an increased hematocrit alone does not imply abnormal renal function. It is suggested that an abnormal glomerular filtration rate, if found in patients with polycythemia vera, expresses the presence of renal disease and not the increased hematocrit alone.

Red Cell Life Span and Iron Turnover in Patients with Hypoxia due to Pulmonary Emphysema

By *James F. Hammarsten, Walter Whitcomb, James Lowell and Philip C. Johnson.* Medical and Radioisotope Services, V.A. Hospital, and Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Wilson et al. have demonstrated that patients with pulmonary emphysema do not show the hematologic adaption to hypoxia characteristic of residents at high altitude. The reason for this failure has not been established, however. Wilson et al. showed it was not related to CO_2 retention and suggested it might be secondary to chronic infection.

Sixteen patients with severe pulmonary emphysema and arterial oxygen saturation below 92.0% were studied. Pulmonary function studies were characteristic of pulmonary emphysema. Eight healthy normal men served as controls.

The hematologic data in the patients with emphysema showed hematocrit 54.1 ± 5.6%; hemoglobin 16.8 ± 1.1 Gm. %; red blood cell count 5.27 ± 0.53 × 10^6 per cubic mm.; MCV 103 ± 5.7 cubic μ; MCH 32 ± 2.1 micro μg.; MCHC 31 ± 2.1%; reticulocyte count 0.95 ± .37%; serum iron 129 ± 47 μg. %; the Cr-51 red blood cell half time was 26.6 ± 6.6 days; red cell mass 48.0 ml./Kg. of body weight; Fe^{59} disappearance 71.1 ± 43.8 minutes; and Fe^{59} reappearance 5.3 ± 3.2 days. None of the group reached the predicted hemoglobin level for its degree of hypoxia; the mean hemoglobin for the group was 88% of the predicted.

The corresponding data for the normals was Cr^{51} red blood cell half time 26.0 ± 3.5 days; Fe^{59} disappearance 74.8 ± 8.78 minutes; and Fe^{59} reappearance 6.4 ± 1.2 days.

The normal red cell life span and normal iron turnover indicated that the failure to adapt fully to the low oxygen saturation is not related to iron deficiency or hemolysis; moreover, the finding of

normal red cell life span and normal rate of Fe^{59} disappearance contrasts with some reports of the hematologic findings in the anemia of chronic infection. The explanation of the phenomenon described must therefore be in the responsiveness of the bone marrow.

Acute Reactivation of Chronic Myeloid Leukemia While on Myleran Maintenance Therapy

By *William L. Wilson, James F. McDonald, Jr. and Charles L. Spurr.* V.A. Hospital, and the Department of Medicine, Baylor University College of Medicine, Houston.

The use of Myleran in the control of the clinical features of chronic myeloid leukemia, including peripheral blood and bone marrow abnormalities, has been previously reported. The several clinics reporting have used either intermittent or maintenance programs of therapy. In our studies, we used primarily a maintenance program with an average dose of 3.0 mg./day. The average duration of disease in this group of 12 cases is now two years. The current status of this group is of particular interest in that 6 patients have developed acute and subacute myeloblastic reactivations of their leukemia, occurring during maintenance therapy in 5 cases and shortly after discontinuing therapy in 1 case. The average period of treatment was 20 months. These reactivations have been associated with gradual splenic enlargement, increasing number of immature myeloid cells—including basophils and eosinophils in the peripheral blood—and anemia. There is evidence of a hemolytic anemia.

Uric Acid Excretion in Patients with Leukemia, Lymphoma, and Related Diseases

By *Shirley L. Rivers and Charles M. Huguley, Jr.* Department of Medicine, Emory University School of Medicine, and the Emory University Hospital, Emory University, Georgia.

In patients with leukemias, lymphomas, and similar diseases, there is often increased excretion of uric acid, presumably due to the excessive rate of production and destruction of blood cells. Various types of therapy may, by increasing the rate of destruction of cells, lead for a time to an even greater rate of excretion of uric acid. A complication of this, in some instances, may be typical gout. A more serious and more common complication is the production of uric acid renal calculi, which may cause urinary obstruction and uremia.

We have made serial studies in patients before and after therapy. All patients were on a diet containing 2000 calories, low in purines, and with 70 Gm. of proteins, or were eating less than this amount. Daily 24-hour urine uric acid determinations were done, and blood uric acid levels were done at appropriate intervals. Salicylates and other uricosuric agents were withheld. A comparison of blood uric acid levels and urinary uric acid excretion in 10 normal controls with 45 patients before and after therapy was made.

It has been suggested that urinary complications might be avoided by the use of low purine diets, forcing fluids, alkalinizing the urine, and by avoiding uricosuric agents. It has also been suggested that therapy be given cautiously in any patient who has a high blood uric acid level or evidence of renal involvement with the primary disease. We agree with these suggestions. In our patients, the most important consideration appeared to be the maintenance of very high fluid intake. One patient was able to excrete a concentration of 150 mg.% in his urine without alkalinization, and another excreted 100 Gm.% concentration of urine uric acid; in such cases the total excretion sometimes varied considerably with the urine output. Several patients in the series excreted over 2000 Gm. per 24 hours without any complications and one put out 8255 mg. in 5540 cc. of urine without renal complications, although his blood urea nitrogen and blood uric acid were temporarily elevated.

In spite of our efforts to maintain an adequate urinary output, two patients with acute leukemia died of renal complications. It would appear that ability of the kidney infiltrated with leukemic cells to excrete a large volume of water is of prime importance.

Microelectrophoretic Studies of Human Blood Platelets

By *S. William Ross.* Department of Medicine, University of Arkansas Medical Center, Little Rock, Arkansas.

Properties of human erythrocytes have been studied extensively by microelectrophoresis but comparable studies of human blood platelets have not previously been reported. Using an Abramson type of horizontal microelectrophoresis cell with a power supply furnishing a constant current (6 milliamp.) and phase microscopy, the mobility of platelets in various solutions was determined. Platelets were obtained from normal personnel and from patients of the University Hospital. All equipment used in obtaining platelets was modified so that surfaces were made nonwettable. Sequestrene was used as the anticoagulant. The ionic strength of the final solution was kept constant and the pH was varied using citrate, phosphate, or protein buffer systems.

The isoelectric point of platelets in citrate buffer was found to be 4.0 with S. D. of 0.08. The isoelectric point in normal human serum is 4.41 with S. D. of 0.11. Further studies were carried out to determine the affect of albumin, gamma globulin and hemoglobin on platelet mobility. The isoelectric point of platelets in albumin was higher than in the citrate buffer, whereas in globulin the isoelectric point was very close to that of serum. Hemoglobin raised the isoelectric point of platelets over 5.0. Heparin lowered the isoelectric point of platelets below 1.0. The effect of change in calcium ion concentration and of washing the platelets will be reported.

CARDIOVASCULAR SYSTEM

Anatomic Research: 1. Clinical Landmarks of the Cardiac Septa

By *Jorge A. Rodriguez.* Department of Surgery and the Surgical Research Laboratory, University of Mississippi Medical Center, Jackson, Mississippi.

The internist and the surgeon have a common stake in lesions which involve the cardiac septa, be they congenital, traumatic, or the result of myocardial infarction. Yet, despite the fact that intracardiac surgery under direct vision is a common reality, it is apparent that many surgeons and internists have directed little attention to the anatomic landmarks and to the most frequent congenital defects. The principal reason for this is that until recently, such information was almost of no practical importance; anatomic studies were purely descriptive and quite incomplete. The purpose of this present investigation was to correct this gap in anatomic data of clinical importance.

Using human hearts and published surgical experience, the following points, among others, have been examined: 1. Relationship of the vestigial valve of the inferior vena cava to the Vieussens ring. 2. Relationship of the coronary sinus ostium to the orifices of the inferior vena cava, posterior leaflet of the tricuspid valve and the fossa ovalis. 3. Relations of the membranous portion of the interventricular septum to the annulus fibrosus of the tricuspid and aortic valves, as well as to the medial leaflet of the tricuspid and right lateral aortic cusp. 4. Relations of the crista supraventricularis to the sigmoideal cusps of the aorta. 5. Boundaries of the septal portion of the pulmonary infundibulum. 6. Blood supply of the septa. 7. Anatomic location of the bundle of His and its distribution. 8. Attachment of the papillary muscle of the arterial conus of Luschka.

Also, a study of the various septal defects was included, focusing attention as to type (classification), number, size, topography and associated malformities.

Continuous Recording of Cardiac Output by the Fick Method

By *Arthur C. Guyton, Charles A. Farish and Ray J. Nichols.* University of Mississippi School of Medicine, Jackson. (Aided by a grant from the American and Mississippi Heart Associations.)

An apparatus has been constructed to record the cardiac output continuously by the Fick method. This apparatus is composed of three separate units: a photoelectric arteriovenous oxygen difference recorder, an oxygen analyzer for measuring the rate of oxygen utilization, and a computer for dividing the rate of oxygen utilization by the A-V oxygen difference to give a continuous recording of cardiac output. Using this apparatus it has been possible to record progressive changes in cardiac output following transfusion of blood, hemorrhage of the animal, injection of epinephrine, and a number of other procedures that affect the circulation. Simultaneous records made by a direct flowmeter in the venous return to the heart and by the present apparatus have shown that the apparatus records quite faithfully. A lag time of less than one minute exists in recording the changes in cardiac output following transfusion or hemorrhage. The continuous recorder has also proved to be valuable for demonstrating which states of the circulation are so unsteady as to make the Fick method unreliable. For instance, such rapid fluctuations occurred in both the A-V oxygen difference and rate of oxygen utilization following injections of epinephrine that the Fick method seemed to be totally invalid for as long as three minutes following an injection.

Cardiac Output in Man Determined by External Counting of RISA

By *B. J. Duffy, Jr., J. R. Howley, J. Cosma and C. A. Hufnagel.* Departments of Medicine and Surgery, Georgetown University Hospital, Washington, D. C.

A 10 microcurie dose of radioiodinated serum albumin (RISA), injected into the antecubital

vein and externally counted in its first transverse through the heart provides, when recorded graphically, a reproducible curve of cardiac blood flow and, by direct extrapolation, a measure of cardiac output in man.

The results of this radioisotope technic have been compared with the direct Fick method of cardiac output in 15 patients, both normal and cardiac. The results of the two methods are comparable within established limits.

The external counting technic was used in a study of 38 patients with aortic insufficiency. In 12 patients cardiac output was determined before and after surgical placement of the plastic (Hufnagel) valve in correction of the aortic insufficiency.

The results agree with the published data of Rose from this clinic on cardiac output and dye injection curves in that the isotope method also implies improvement in cardiac function following valvuloplasty.

The practical advantage of the external counting technic for cardiac output measurement is its ease, safety and reproducibility.

Restenosis of the Mitral Valve: A Hemodynamic Study

By *Joseph M. Merrill and Walter G. Gobbel, Jr.* Research Laboratory and the Medical and Surgical Services of Thayer V.A. Hospital, and the Department of Medicine and Surgery, Vanderbilt University School of Medicine, Nashville, Tennessee.

Three patients who had mitral valvotomy developed physiologic evidence of restenosis of the mitral valve. This has been determined by cardiac catheterizations at yearly intervals. Direct palpation or inspection of the valve at subsequent surgery or autopsy has given proof of restenosis. Following valvotomy, one patient had a good subjective improvement with hemodynamic data suggesting that a technically successful valvotomy had been performed. By the end of 2 years symptoms had returned, accompanied by a rising pulmonary vascular resistance. A second valvotomy lowered the pulmonary artery pressure and has been accompanied by marked subjective improvement. A second patient developed subjective improvement immediately following valvotomy but this was not associated with a fall in pulmonary vascular resistance. Fifteen months later progressive disability with increasing pulmonary artery pressure indicated that a second operation was in order. Bacterial endocarditis following left heart catheterization prevented further surgery. At autopsy, the previously widely split valve had refused, so that the orifice was less than one cm.^{2} The third patient showed subjective improvement immediately following the first operation. Catheterization studies at yearly intervals over the next two years failed to confirm improvement. Increasing dyspnea led to a second operation. This was followed by both subjective improvement and a striking fall in pulmonary artery pressure. It is therefore considered that restenosis of the mitral valve occurs and that the resulting increased pulmonary vascular resistance is partially reversible by a second valvotomy.

Comparison of the Hemodynamics in Experimental Mitral Stenosis and Mitral Stenosis with Azygos Vein Ligation

By *Albert L. Hyman and William E. Jaques.* Departments of Pathology and Medicine, Louisiana State University School of Medicine, New Orleans.

Conflicting reports have appeared concerning the altered pressure relationships in the pulmonary vascular system following acute constriction of the mitral valve. During the course of studying the pulmonary hemodynamics in dogs with experimental mitral stenosis, we became impressed with the lack of the expected degree of pulmonary hypertension. It appeared that a likely explanation might lie in the development of shunts between the pulmonary and bronchial circulations. To test this hypothesis, 9 dogs were subjected to an experimental mitral stenosis and compared with 11 dogs with an experimental mitral stenosis and azygos vein ligation. A high azygos vein ligation obliterates any shunts into the bronchial venous system.

The two groups of dogs with mild chronic stenosis were studied with cardiac catheterization following acute constriction of the mitral valve area. This study was supplemented by lung biopsies before and after constriction in 2 dogs of each series.

The control pressures in the azygos ligated group were slightly higher than in the intact azygos group. Following acute mitral constriction, the pulmonary wedge pressure in the intact azygos group rose an average of 14 mm. Hg, while pressures in the azygos ligated group rose an average of 26 mm. Hg. The pulmonary artery pressure rose 6 mm. Hg in the intact azygos series, but increased 20 mm. Hg in the azygos ligated series. The total resistance to flow at the pulmonary artery level rose 570 dynes cm.^{5} in the intact group, whereas that resistance rose 2000 dynes cm.^{5} in the ligated group. There was a fall of 140 dynes cm.^{5} in the pulmonary arteriolar resistance in the intact series,

while the ligated series produced an increase of 215 dynes cm.^{5} The cardiac output fell in both groups.

Lung biopsies in both groups were similar. There was a paucity of transudation into the alveolar spaces and no structural vascular changes. Interstitial edema was more prominent in the azygos ligated group.

The failure of the intact azygos group to show substantially elevated pressures is attributed to drainage of much of the postconstriction blood volume into the bronchopulmonary anastomoses.

Observations on the Mechanism of Gallop Rhythm

By *J. J. Leonard, A. M. Weissler and J. V. Warren.* Department of Medicine, Duke University School of Medicine, and the V.A. Hospital, Durham, North Carolina.

Physiologic studies have indicated that both auricular (presystolic) and ventricular (protodiastolic) gallop sounds are related to intracardiac pressure wave phenomena. Studies on these sounds have been undertaken utilizing the effect of blood pooling by venous occlusion cuffs, a procedure that has been demonstrated to alter intracardiac pressure. Logarithmic phonocardiographic and apex cardiographic recordings were obtained in patients with gallop rhythm before, during and after application of venous cuffs.

Eight patients with ventricular gallop sounds were studied. In 6, the sound disappeared and returned with application and release of cuffs. The apex cardiogram demonstrated corresponding changes. In 15 patients with auricular sounds, not only did the cuffs produce a reduction of the sound in 8, but also a temporal migration of the sound toward the first sound in all. In 5 patients with an opening snap of the mitral valve, tourniquets produced no effect on either amplitude or timing.

These observations on the effect of a procedure which predominantly alters the levels of intracardiac pressure, rather than pressure gradients, provide additional evidence on the genesis of gallop sounds. They further suggest a simple clinical test in the differentiation of ventricular gallop and third sounds from the opening snap of the mitral valve.

The Prevention of Ventricular Fibrillation Following Acute Coronary Arterial Occlusion by Blockade of the A-V Node

By *Watts R. Webb and Samuel E. Field, Jr.* Department of Surgery, University of Mississippi School of Medicine, Jackson.

The basic mechanisms of ventricular fibrillation have not as yet been clearly elucidated. The incidence following various stimuli can be altered by numerous drugs, electrolytes, hormones or interruption of sympathetic impulses.

The present study concerns the incidence of ventricular fibrillation as affected by factors altering the conduction system of the ventricle. In mongrel dogs, the A-V node has been blocked with procaine to produce complete A-V dissociation. In this preparation a faradic current, which will always produce fibrillation in control animals, would only rarely produce fibrillation in the presence of an A-V nodal rhythm. If an idioventricular rhythm were produced, fibrillation never occurred.

This study has been extended to observations of arrhythmias following total left coronary artery occlusion, which presents a maximal stimulus. In 8 control animals, ligation of the left coronary artery at its origin produced ventricular fibrillation in 30 to 150 seconds. However, after anaesthetization of the A-V node, only one of 14 dogs fibrillated early. Two fibrillated after 7 and 8 minutes, respectively, and in the remaining 11 the ventricles very gradually stopped but were not fibrillated.

Pulmonary Dynamics in the Presence of Atresia of One Pulmonary Artery

By *H. Stephen Weens, John R. Derrick and Albert H. Wilkinson, Jr.* Department of Radiology, Emory University School of Medicine and Grady Memorial Hospital, Atlanta.

One of the rare causes of pulmonary emphysema is partial or total atresia of the pulmonary artery of the opposite lung. This condition is of importance in the differential diagnosis of other causes of pulmonary emphysema as well as atelectasis of the affected side. The clinical diagnosis of this condition depends upon fluoroscopic observation of diminished pulmonary function and absent or diminished pulmonary artery pulsations on the involved side. Diagnostic confirmation of the condition is obtained by angiocardiography.

Two patients exhibiting varying degrees of pulmonary artery atresia have been studied by the technic of cineroentgenography. This procedure permits satisfactory demonstration of deficient function of the affected lung associated with the development of pulmonary emphysema and mediastinal herniation on the unaffected side.

Dynamics of Ejection Murmurs of the Pulmonary Artery

By *A. Calhoun Witham.* Department of Medicine, Medical College of Georgia, Augusta.

Study of PCG's, usually with simultaneous in-

tracardiac pressures, in a wide variety of conditions revealed that configuration of the pulmonary systolic murmur was more closely related to the pattern of right ventricular ejection than to any particular disease. The basic ejection murmur found in normals has reached peak intensity by the end of the first sound so that decrescendo vibrations exist during the first half of systole. This follows the classic concepts of an early systolic velocity peak in normal circulation. With increased stroke volume (exercise, anemia, atrial defect, etc.), intensity and duration of the vibrations increase but configuration is unchanged. The consequences of pulmonary hypertension (primary, congenital lesions, mitral stenosis) are to delay onset of ejection by prolonging isometric contraction and to extend the ejection period. Acoustically, this tends to bring the murmur peak nearer midsystole, to extend its duration, and to make the pulmonary valve closure sound late. With valvular pulmonic stenosis, isometric contraction is normally short because of low pulmonary diastolic pressure, but maximal velocity develops slowly so that early vibrations are minimal and the highest intensity much later. This lateness and the duration of right ventricular systole correlate with the degree of stenosis. Murmurs of infundibular stenosis appear quite different, beginning loud and early with a decrescendo configuration. Several theories exist but the paucity of accurate simultaneous pressure records makes explanations tentative. The systolic murmur of pulmonary insufficiency seems fundamentally due to increased volume and velocity of ejection. Isometric contraction is short if the pulmonary diastolic pressure is low and the murmur appears early. Although the systolic murmur is similar in free regurgitation with idiopathic aneurysmal dilatation, a striking delay in the rise of the pulmonary artery pressure is present, probably related to the abnormal stretch properties and capacity of the vascular bed in this disease.

Clinical Measurement of Circulation Time: A Comparison of Magnesium Sulphate and Evans Blue Dye in Normal Subjects

By *Tom M. Dees, John A. Rumsfeld, William F. Miller and Carleton B. Chapman.* Cardiopulmonary Laboratory of the Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

The relation of the circulation time (CT) as measured clinically (with Decholin or $MgSO_4$) to the concentration of the test substance needed to elicit a response at the receptor site (tongue or pharynx) has not been defined. To settle the point, a mixture of T-1824 (10 mg.) and $MgSO_4$ (one ml. of 50% solution) was injected into the antecubital veins of 39 normal subjects a total of 71 times. The point of injection and the time at which the patient responded to $MgSO_4$ were marked on the photographic record of the oximetrically recorded dye curve. It was found that CT regularly occurs about 3.5 seconds after the appearance time (AT) and about 6.5 seconds before the peak time (PT) of the dye curve. The mean values were: CT 17.9 ± 5.5 seconds, AT 14.5 ± 5.5 seconds, and PT 24.3 ± 8.2 seconds.

Variance analysis disclosed that CT and AT are highly repeatable within individuals but that there is a large interindividual variation. The influence on the latter of age and sex appears to be significant. Venous pressure, taking intrathoracic pressure into account, had no influence on the time intervals, nor did total body weight or surface area.

It is concluded that in normal subjects, CT represents a time interval which is longer than that at which the first portion of the test substance begins to arrive at the receptor site, but which is considerably shorter than that at which maximal concentration is attained. Age and sex appear to be important determinants of the wide range for CT found in normal subjects.

Hypothermia as an Adjunct to Total Cardiac Inflow-Outflow Occlusion in the Dog

By *Dale Dominy, James E. Thoroughman, William Wansker and John M. Howard.* Department of Surgery, Emory University School of Medicine, Atlanta.

This investigation has been aimed at determining the maximum time of total occlusion of the aorta and vena cavae, compatible with life when utilizing the adjunct of hypothermia. Thirty dogs were studied. Operations were performed under sodium pentobarbital anesthesia and controlled respirations. Cooling was affected by an ice-water bath at temperatures varying from 30–26° C. and were divided into groups in which the inflow-outflow occlusion of the heart was maintained for 5, 10, 15 minutes, respectively. Electrocardiograms were recorded intermittently throughout the entire experiment.

All dogs occluded for 5 and 10 minutes lived, with the exception of two animals whose deaths were attributed to technical errors. Twenty dogs underwent occlusion for 15 minutes each, 9 of which survived. Four of the dogs that died had complications at the time of surgery such as an aortic per-

foration, and one dog of the remaining 7 that died lived seven hours.

The dogs that survived in group 3 had rectal temperatures during occlusion varying from 22.2 to 26.7, whereas the dogs that died had rectal temperatures above 26.7. Changes in electrolyte concentrations of serum from the inferior vena cava before, during and after release of the vena caval occlusion revealed no correlation with survival. The majority of the dogs that survived the 15-minute period of total vascular occlusion of the heart exhibited a reversible ventricular fibrillation.

Limbic System Seizures and Blood Pressure Responses

By *O. Andy, R. Chinn and P. Bonn.* Laboratory of Experimental Behavior, Division of Neurosurgery, University of Mississippi Medical Center, Jackson. (Aided by a grant from the U.S.P. H.S.)

Behavioral, electric and blood pressure changes were correlated during after-discharges induced by electric stimulation of various limbic system structures.

Procedure: In 20 awake cats Hess electrodes were placed in various subcortical and cortical structures. Blood pressures were recorded with a Sanbourne Polyviso and Statham transducer from a cannulated femoral artery. Local ½% Novocain was employed for surgical anesthesia. Grass electroencephalograph and stimulator were used for electrical recording and stimulation, respectively. Electrical stimuli consisted of 30 per second frequency, 1 millisecond pulse, 5 seconds duration and 1½ to 5 volts. Voltages most frequently employed were 1½ to 3 volts. After the completion of each experiment, the brain was profused with saline and formaldehyde solutions. Points of stimulation and recording were then identified on serial histologic sections. Over 300 different points were stimulated, from which over 1500 seizures were elicited while blood pressure and electroencephalographic recordings were made.

Results: Stimulation of the amygdala and periamygdaloid area invariably produced a drop in the diastolic and systolic blood pressure. During the after-discharge the pressure drop varied from 5 to 30 mm. of mercury. The duration of the drop was usually much longer than the duration of the seizure—in most instances twice as long. During these discharges there were ipsilateral facial movement, mastication and pupil dilatation. Subthreshold stimuli also produced blood pressure depressions, but these were not as pronounced as those associated with an induced after-discharge.

It is interesting to note that a primarily induced hippocampal discharge usually does not produce blood pressure changes even if the discharge lasts 4 or 5 times as long as the average amygdaloid seizure. Only an occasional slight depression was observed during its termination. However, the hippocampus having a propagated amygdaloid after-discharge has a tendency to counteract the blood pressure drop which usually accompanies amygdaloid seizures.

Cingulate gyrus stimulation and after-discharges did not consistently produce blood pressure changes, although an occasional slight and temporary decrease was noticed.

Septal after-discharges were much more inclined to produce blood pressure alterations than hippocampal after-discharges. Its after-discharges were usually accompanied by a depression of blood pressure. These changes could not be correlated with involvement of the amygdala or hippocampus, both of which are anatomically associated with the septum.

Effect of DOCA Upon the Development of Reno-Prival Hypertension

By *H. G. Langford, J. R. Snavely and Don M. Turner.* Departments of Medicine and Surgery, University of Mississippi School of Medicine, Jackson.

The role of the adrenals in the development of reno-prival hypertension has not been completely settled, nor has the manner in which DOCA produces hypertension. The present experiments were designed to see if the rate of development of reno-prival hypertension was affected by administration of DOCA. Unilateral nephrectomy was done upon mongrel dogs and the remaining kidney was removed two weeks later. After nephrectomy, the dogs were maintained on 1% saline as drinking water and given a commercial dog food. Alternate dogs were given DOCA in a total dose of 50 mg. over the first three days. Control blood pressures and determination of blood corticoids were done. These observations were repeated on the fourth day. A total of 14 dogs was studied. In the DOCA-treated animals there was a mean rise in blood pressure of 53 mm. Hg. and in the control animals a drop of 3 mm. Hg. These differences are statistically significant ($P > .01 < .02$). Results from the steroid determinations are available in four animals to date, in all of whom there was a marked drop in blood steroid concentration. There was no significant difference in

weight gain in the two groups. These results show that the kidney is not necessary for the development of steroid hypertension. Though they do not disprove the contention that the adrenals have nothing to do with reno-prival hypertension, they suggest that this may not be necessarily so. The marked drop in blood corticoids cannot be easily explained, but would be compatible with the adrenals shifting over to the production of aldosterone due to the disordered electrolyte balance.

Factors Influencing Development of Hypertensive Vascular Disease in Rats with Renal Injury

By *Alvin P. Shapiro and Julian Melhado.* Department of Internal Medicine, University of Texas, Southwestern Medical School, Dallas.

In order to evaluate factors which may influence hypertensive vascular disease in the rat, renal function was measured in animals who had been subjected to figure-eight ligature of the kidney and contralateral nephrectomy. In addition, the rate of development of hypertension and its severity were studied when operated-on rats were exposed to the stress of a chronic behavioral disturbance.

Seventeen rats who survived an average of 36 weeks after surgery were studied. All became hypertensive, with average systolic blood pressure of 146 ± 14 mm. Hg. and average systolic maximum of 160 ± 16. BUN was 52 mg.%; PSP excretion, 50%; osmolar concentration, 1773 milliosmoles/ml. in 48 hours. (Normals = 29 mg.%, 63%, and 2413 milliosmoles/ml., respectively; p = .001.) Impaired PSP excretion and osmolar concentration both correlated with elevation of BUN, but not with each other. The ligated kidney hypertrophied but failed to compensate for impaired function; actually, a negative correlation was demonstrated, in that animals with the most hypertrophy had the worst functional impairment. Level of blood pressure did not correlate with any of the changes in renal function or with renal mass.

The 17 animals included 9 that were "psychologically stressed" by a specifically designed conditioning procedure, and 8 that were untreated. Although levels of blood pressure in the two groups were not significantly different, the onset of hypertension after surgery was sooner, its duration longer, and the severity of the disease, as evidenced by renal function, heart size, and adrenal size, increased by the behavioral disturbance. These differences were significant statistically.

The data thus demonstrate that significant impairment of renal function is present in rats with renal hypertension, but that its extent does not correlate with blood pressure levels. Renal hypertrophy does not compensate for functional impairment nor does it prevent hypertension. In addition, stress of a behavioral disturbance may enhance the hypertensive mechanism.

The Protective Effect of Blood Pressure Control on the Vascular Deterioration Associated with Hypertension

By *Jack Burgess, Ralph Ford, Keith Pevey, Charles Heider, Robert Bloodwell and John Moyer.* Baylor University College of Medicine, Houston.

There remains a question as to the effectiveness of blood pressure reduction in arresting the vascular deterioration associated with hypertension. Two years or more is an adequate period of time to evaluate some effects of therapy in patients with severe hypertension as compared with untreated patients with hypertension of an equivalent severity. The results in our clinics have been evaluated as to clinical response in the important vascular beds, i.e., brain, heart and kidney, which areas are damaged most frequently in patients with severe hypertension. Of particular quantitative significance are the observations made on renal function, i.e., glomerular filtration rate and renal blood flow, before and after treatment in both the treated and the untreated patients. The clinical results indicate that reduction in blood pressure was accompanied by a significant decrease in both morbidity and mortality. The observations on renal function indicate that the patients fell into two groups, those with mild disease and those with severe disease. In the patients with mild hypertension renal vascular deterioration was not significantly greater in the untreated patients than in the treated ones. However, in the patients with severe disease renal vascular deterioration was quite marked over the two-year period. Treatment had a definite protective effect in arresting renal vascular deterioration. If uremia was present, therapy was ineffectual since adequate blood pressure reduction could not be accomplished without aggravating the renal failure.

Experimental and Clinical Evaluation of Tetrahydrozoline, a Pressor Amine Showing Hypotensive Properties.

By *Frank A. Finnerty, Jr., Joachim H. Buchholz and Robert L. Guillaudeu.* Georgetown University Medical Division, District of Columbia General Hospital, Washington, D. C.

The recent interest in serotonins and antiserotonins has renewed our interest in a substance with hypertensive and hypotensive properties, DL

2-(1,2,3,4-tetrahydro-1-naphthyl)-imidazoline (tetrahydrozoline), a pressor amine closely related chemically and pharmacologically to naphazoline.

Rapid intravenous administration of 0.5 to 1 mg. Tetrahydrozoline in 42 patients was followed in 30 seconds by a transitory asymptomatic pressor response characterized by a 34% average increase in mean arterial pressure and a 32% average decrease in pulse rate. Twenty-five to 30 minutes following the initial injection, a hypotensive phase ensued which lasted an average of six hours and was characterized by a 40% average decrease in mean arterial pressure and a 14% average decrease in pulse rate.

Hemodynamically (8 patients), the pressor phase was characterized by a 36% average fall in cardiac output (indicator dilution technic) and a 58% average increase in peripheral resistance. At the peak of the hypotensive phase, a 25% average decrease in cardiac output and an 11% average decrease in peripheral resistance were noted.

No pressor response followed the slow intravenous injection of 20 cc. of Tetrahydrozoline in a concentration of 0.01 mg./cc. Twenty-five minutes following injection, however, a 17% decrease in mean arterial pressure and a 20% decrease in pulse rate were observed. In six hours, when the mean arterial pressure had returned to control levels, a repeat injection of 2 mg. of Tetrahydrozoline undiluted was followed by a 24% rise in mean arterial pressure.

When Tetrahydrozoline was given orally in a dosage of 4 to 10 mg./day, no pressor response was noted. Twenty-seven of 34 patients exhibited an average reduction of 44 mm. Hg systolic and 26 mm. Hg diastolic pressure and an average decrease in pulse rate of 20 beats per minute. Drug resistance developed in 7 to 10 weeks. No significant change in cardiac output and renal blood flow followed oral administration. The bradycardia usually could be abolished by atropine.

It would seem that the transitory hypertension following Tetrahydrozoline, caused by a high drug concentration in the blood, hemodynamically resembles norepinephrine. The longer hypotensive phase caused by a minimal drug concentration in the blood hemodynamically resembles the depressor action of epinephrine. The lack of a pressor response following oral administration is best explained by the lack of attaining a high blood concentration.

Although some undesirable central effects will limit the usefulness of Tetrahydrozoline, it would seem that further investigation of other Imidazoline derivatives are in order for the purpose of uncovering a clinically useful and potent hypotensive agent.

Study of Treatment of Essential Hypertension

By *Raymond F. Grenfell.* Department of Medicine, University Medical Center, Jackson (Aided by a grant from Sandoz Pharmaceuticals.)

In January, 1956, a double-blind study was begun employing four different ampules, labeled CA, CB, CC and CD. Two groups of ampules contained normal saline and two groups contained a mixture of .1 mg. dihydroergocornine, .1 mg. dihydroergokryptine and .1 mg. dihydroergocristine produced commercially as Hydergine.

Prior to the administration of these substances intramuscularly, a complete physical examination, including complete blood count, serology, urinalysis, blood glucose, blood creatinine, blood nonprotein nitrogen, phenolsulphonphthalein excretion determination, chest x-ray for heart size and electrocardiogram, were done. Also, phentolamine intravenously was given to determine the presence or absence of a pheochromocytoma. Following these studies, those patients who fall into stages I, II and III of the Schroeder classification are being used. All patients of this series are ambulatory and receive no other medicine. The control blood pressure must be greater than 150/100 to be included in this study. The blood pressure is recorded at 10-minute intervals for a period of 30 minutes. The lowest systolic blood pressure reading for each visit is the one used in analyzing the effect of the injections. All blood pressure readings are being made with the patient in a sitting position.

A specific dosage schedule is being used as follows: 3 injections a week for two weeks; 2 injections a week for two weeks; 1 injection a week for two weeks; 1 injection every ten days for two injections; 1 injection every two weeks as maintenance dosage.

To date, this study includes 46 subjects who have been under treatment for periods varying from 3 to 27 weeks. These patients are approximately equally divided so that one-half are receiving normal saline and one-half are receiving the drug.

A statistical evaluation has shown no significant difference ($p \leq 0.05$) between the patients receiving the drug and those receiving saline. An analysis of the fall in blood pressure has shown a significant drop in both the systolic and diastolic blood pressures in the saline-treated patients.

Thus, although a blood pressure drop occurs using the drug with a dosage schedule previously described and reported by me in a series of private patients, a similar blood pressure drop occurs and is maintained with the use of normal saline.

Pressure Pulses Recorded as Velocity and Acceleration of Pressure Changes

By *John J. Neal, Jr. and T. J. Reeves.* Department of Medicine, Medical College of Alabama, Birmingham.

The usual analysis of pressure pulse is limited to time pressure correlations at isolated points on the curve. Such a method of analysis has basic limitations because (1) the number of peaks, nadirs and sharp breaks on the curves, which are used in timing and contour analysis, is limited; (2) in abnormal states, pressure pulses often show slurred upstrokes and broad notched areas, making recognition of the routinely measured points difficult; (3) this type of analysis furnishes little direct quantitative data about the variations in slope and contour of the curve.

An electronic differentiator has been employed so that intravascular pressure pulses can be simultaneously recorded as (1) pressure displacement (mm. Hg above the baseline), (2) velocity of pressure change (mm. Hg/second change plotted against time), (3) acceleration of pressure change (mm. Hg/second/second change plotted against time). These rate functions allow more precise definition of the points of the cardiac cycle and permit more rational interpretation of the pressure pulse. In addition, by recording calibrated velocity and acceleration pulses, quantitation of the slope changes seen in the familiar pressure pulse traces may be obtained.

Pressure pulses and rate functions of the ascending aorta of dogs have been studied under the influences of epinephrine, norepinephrine, hypervolemia, and hypovolemia. Calibrated acceleration and velocity pulses enabled quantitation of slope changes occurring under such influences and revealed significant changes before obvious changes are noted in the familiar pressure pulses. These changes appear to reflect changes of myocardial function in a sensitive manner.

Arteriographic Findings in Peripheral Arterial Embolism: Experimental and Clinical Observations

By *William D. Logan, Jr. and William J. Goudelock.* Department of Surgery, Emory University School of Medicine, Atlanta.

Arteriography and aortography are being used with increasing frequency as adjuncts diagnosis of vascular disease. In patients with peripheral emboli, prompt diagnosis and treatment is paramount. Several reports have described these procedures—which they advocated—as accurate in localizing the point of obstruction. Such localization aids materially when surgical removal is contemplated.

On the basis of experience encountered in this hospital, it was postulated that arteriography might not always be accurate.

In the further evaluation of this problem, six dogs were used, with radiopaque emboli being placed in the aorta and allowed to move distally to obstruct major vessels in the lower extremities. Aortograms were then taken which, in each instance of complete obstruction, revealed a segment of artery proximal to the obstruction that did not fill with dye. This phenomenon is interpreted to indicate that sufficient concentration of the contrast medium for roentgenological visualization is not present due to a "static" column of blood which exists in a vessel between the point of obstruction and the last significant proximal branch.

Clinical experience confirms the experimental observation. The "static" column of blood has led to false localization of emboli by arteriography with resulting errors in surgical exposure.

The Arterial Pressure Response to the Valsalva Maneuver as an Index of Peripheral Vasoconstrictor Reactivity

By *Stuart Bondurant.* Aero Medical Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

Normal arterial pressure response to the Valsalva maneuver includes a post-Valsalva overshoot of arterial pressure associated with reflex peripheral vasoconstrictor activity. It has been suggested that decreased pulse pressure during the maneuver is the stimulus which elicits this response. If this is the case, the ratio of stimulus to response of each individual would be an easily determined index of peripheral vasoconstrictor reactivity.

The present study was designed to investigate in the individual subject the correlation between post-Valsalva overshoot and the arterial pressure changes occurring during the Valsalva maneuver. Arterial pressure was measured directly as each of five normal subjects performed a series of 10 to 15 Valsalva maneuvers of ten seconds duration. A series of different oral pressures was used, increasing in 10 mm. steps from 10 to 90 mm. Hg. To establish an unrelated estimate of peripheral vasoconstrictor reactivity, blackout threshold of each subject was determined on the human centrifuge. Individual tolerance to increased gravitational force is known to

depend largely upon peripheral vasomotor reactivity.

In each of the five subjects there was a highly significant positive correlation between the change in pulse pressure from resting to end Valsalva levels and the magnitude of the post-Valsalva overshoot (individual correlation coefficients between .699 and .924). There was no correlation between changes in mean pressure and overshoot. The ratio of systolic pressure overshoot to pulse pressure change of each individual correlated well in these five subjects with blackout threshold. This data strengthens the suggestion of other investigators that the reflex peripheral vasoconstriction associated with the post-Valsalva overshoot of arterial pressure is elicited by decreased pulse pressure during the Valsalva maneuver. The ratio of systolic pressure overshoot to pulse pressure change is an index of individual peripheral vasoconstrictor reactivity, which should have application in clinical practice and as a research tool.

The Role of Regional Acidity in the Production of Irreversible Shock

By *Jack W. Crowell and Lester Bumgarner*. University of Mississippi Medical Center, Jackson. (Aided by a grant from the American and Mississippi Heart Associations.)

If the circulation to a tissue decreases or ceases entirely, the quantity of acidic aerobic metabolites per unit volume of capillary blood increases. Anaerobic metabolism would cause additional acidity. We have found that this decrease in pH causes the formation of thrombi and emboli, possibly by precipitating hemoglobin, which then causes the erythrocytes to become "sticky." This leads to the idea that acidity in any region of the body could cause death.

To show that regional acidity is lethal, concentrated lactic acid was injected into dogs. When acid was injected at the rates of .040–.070 mEq./min./Kg. into the fast-moving aortic blood, an average of 315 minutes was required to kill the animal; injections at the same rates into blood flowing slowly from the femoral arteries through a tube to the femoral vein caused death in 89 minutes (average). Acid injections at the rate of .015–.035 mEq./min./Kg. into the aorta caused no deaths within 500 minutes; however, injections into the slow-moving external circulation caused death in 115 minutes (average). Since the rate of injection into the dog is the same in both experiments, the major effect is the decrease in pH of the blood.

In another experiment, the erythrocytes were removed from the blood of a dog, and then the dog was subjected to one hour and fifteen minutes of circulatory arrest. The erythrocytes were restored and the animal resuscitated. One animal maintained a normal blood pressure for over three hours, but later died. Indications are that the damaging effect of anoxia may be prevented if the erythrocytes are not present during the anoxic period

Acute Exposure to a Moderately Cold Environment. Influence on Venous Pressure in the Superficial Veins of Normal Young Men and Patients with Chronic Congestive Heart Failure

By *G. E. Burch and John Phillips*. Department of Medicine, Tulane University School of Medicine and Charity Hospital of Louisiana, New Orleans.

Hot, humid environment was shown to increase systemic venous pressure in man with and without chronic congestive failure and to precipitate cardiac failure. The observation that cold precipitated acute left ventricular failure in a patient with impairment of cardiac reserve prompted this study of the influence of cold upon venous pressure of normal man and patients with congestive failure and generalized systemic venous hypertension.

Twenty-eight normal men and 15 patients with chronic congestive failure rested in bed in a comfortable room, with pelvic region covered. Venous pressure was recorded with the phlebomanometer before and during applications of pressure over hepatic area. Subjects were then transferred to cold room (12° C. and 56 to 90% R.H.), and measurements were repeated after they became chilled.

Venous pressure was increased appreciably by cold in all except one normal subject, especially with pressure over hepatic area.

Although venous pressure did not rise in response to cold in all patients with failure, it increased considerably in individual instances and produced acute exacerbations in left ventricular failure. Pressure over hepatic area increased venous pressure further.

Superficial veins of all subjects constricted, with probable detrimental effects on cardiac function. Comfortable, pleasant atmosphere is preferable for cardiac disease.

A Study of Postural Effects on Pressure Relationships in the Venous Circulation

By *H. O. Sieker and O. H. Gauer*. Department of Medicine, Duke University School of Medicine and V.A. Hospital, Durham, North Carolina.

The development of a miniature manometer in the leading end of a catheter makes it possible to

measure intravascular pressures without the uncertainty of external reference levels. This is an ideal device for measuring postural effects on venous pressure. Intravenous catheterization was done in 7 normal subjects and 5 patients, 2 with postural hypotension and 3 with congestive heart failure. The manometer catheter was withdrawn through the abdominal and thoracic vena cava at 5 cm. increments and pressure measurements were made at each point with the subject recumbent, head-up, and head-down.

In recumbent normal subjects no appreciable pressure gradient was observed in the abdominal vena cava. At the diaphragm the mean pressure dropped abruptly, but remained positive (3 to 5 cm. H_2O) through the thorax. With head-up tilt, a pressure gradient was noted in the vena cava with a fall from 20–25 cm. H_2O at the iliac veins to a negative pressure of 5–8 cm. H_2O in the upper superior vena cava. The pressure was zero at the right auricle. Ten cm. below the diaphragm the pressure was 12 cm. H_2O and did not vary with position change. With head-down tilt, the mean central venous pressure always increased, but did not exceed the pressure observed 10 cm. below the diaphragm.

In postural hypotension pressures were similar to those in normal subjects. At all points in the venous system and with all body positions, pressures were higher than normal in heart failure patients. The point of unaltered pressure, however, was at the same location as in normal subjects and was 20–30 cm. H_2O. Above this site the pressure fall with change from recumbent to standing was about twice the normal.

This study, which has established the pressures throughout the vena cava in various body positions, aids in explaining the role of venous pressure in postural adjustments of the circulation in normal and certain disease states.

ENDOCRINES AND METABOLISM

In Vivo Estimation of Lean Body Mass

By *Edward J. Werdein, Richard J. Meyer, Marcus Schaaf and Laurence H. Kyle.* Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Measurement of body composition by either densitometric or volume distribution technics is dependent upon relative constancy of the lean body mass (LBM). This is best evaluated by measuring the percentage of water in LBM by simultaneous determination of body density and total body water. Such studies in animals have revealed values that check satisfactorily with carcass analysis. Limited studies in humans have demonstrated similar average values for the percentage of water in LBM, but the spread has appeared greater than can be explained readily by methodologic errors. This study was conducted to provide more data on simultaneous methods for in vivo corporeal dissection and to explore the possibility that variation in the proportion of mineral mass might account for this range of variation.

In normal subjects and patients with Cushing's syndrome, body density and then LBM was obtained by underwater weighing. Total body water (TBW) was measured by antipyrine dilution, checked in some instances by D_2O or radio-antipyrine spaces. In 25 normal subjects, water in LBM averaged 68% but the range was from 54.0 to 75.7%, most subjects showing values below the accepted norm of 72%. In five patients with Cushing's syndrome, water in LBM averaged 98.8% with a range of 81.6 to 123.4%. In two patients without detectable osteoporosis water averaged 84.6%, but in three patients with decreased bone density the implausible average of 108.3% was noted. Serial compositional studies were continued in two of these patients after surgical therapy. In one without osteoporosis the percentage of water in the LBM approached normal within two months and it appeared that the original abnormality was mainly attributable to overhydration. In the other patient, who had severe osteoporosis, the marked elevation of the percentage of water in LBM and its slow reversion to normal indicated both overhydration and decrease in density of the LBM.

These data indicate that variability in the density of the LBM in Cushing's syndrome is attributable to both overhydration and loss of mineral. They suggest significant variation in composition of the LBM in normal subjects, possibly attributable to differences in proportionate skeletal mass.

Studies of the Satiety Response in Mice

By *Guy Hollifield and William Parson*. University of Virginia School of Medicine, Charlottesville.

Satiety represents the full or partial satisfaction of food drive and as such is an important factor in the regulation of food intake. The increase in activity of fasted animals is well known. Equally striking is the decreased activity on refeeding.

These studies have been concerned with (1) whether this decrease in activity is truly a satiety response and (2) if so, what factors are necessary to provoke a satiety response. In order to test the first premise, a large number of mice were deprived of food for 24 hours and then fed with commercial chow. During the period of starvation the level of spontaneous running activity in a wheel invariably rose above the control period activity and fell to about 20% of control period activity on feeding. Mice starved for four days maintained activity levels well above the base line. Mice with lesions in the hypothalamus, which produced hyperphagia and obesity, did not change their activity levels on feeding. Attempts to modify the response to starvation and feeding with measures which changed the activity levels of mice during ad libitum feeding, such as oöphorectomy, and the administration of thorazine and reserpine did not prevent the appearance of either the food drive or satiety response.

The satiety response is not dependent on the overeating which usually follows a period of fasting, since it was provoked by less than the average daily intake of food. A satiety response occurred following the ingestion of either lard, sucrose or casein. Kaolin sweetened with saccharin did not produce a satiety response although it was consumed in amounts nearly equal to either lard, sucrose or casein.

From these studies it appears that the decrease in spontaneous running activity on refeeding after a fast is a satiety response and that this response is not the result of the act of eating or the presence of material in the gut, nor is it completely dependent on the amount consumed. This response seems to require the consumption of at least a small amount of simple food stuff. Studies to clarify further the nature of the satiety response are now in progress.

The Acute Effects of Estrogens on Radioactive Iodine Uptake of Hypothyroid, Euthyroid and Hyperthyroid Patients

By *F. Ozer, L. G. May, A. Bennett, J. E. Johnson, Jr. and R. Gregory*. Department of Medicine, University of Texas Medical Branch, Galveston.

Older clinical studies on the effect of estrogen on human thyroid function were limited to the use of basal metabolic rates. Recently, it has been shown that estrogen administration to euthyroid patients causes increase of the serum precipitable iodine.

This is a study of the effect of conjugated estrogen (Premarin) and a synthetic nonsteroid estrogen, chlorotrianisene (Tace), on thyroid function as measured by uptake of I^{131} in hypothyroid, euthyroid and hyperthyroid patients. All patients studied were females in the childbearing and postmenopausal years.

After control values were established for both groups, I^{131} uptake studies were again made on the eighth day of Premarin (1.25 mg. t.i.d.) and Tace (12 mg. q.i.d.) administration.

Studies on 6 hyperthyroid patients showed a significant fall in thyroid clearance and 30-minute and 24-hour uptake of I^{131} after Premarin therapy. Subjective and objective clinical signs of remission of the hyperthyroid state paralleled changes in I^{131} studies in all 6 patients. Sixteen euthyroid patients who received either Premarin or Tace and 7 hypothyroid patients who received Premarin showed no significant change in I^{131} measurements or in clinical status in this short-term study.

Studies involving the prolonged use of estrogens in hyperthyroidism are in progress.

Adrenocortical Function in Myxedema

By *Gerald A. Williams, K. R. Crispell and William Parson*. Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville.

There is a prevailing concept that adrenocortical function is decreased in myxedema. This is based on decreased urinary 17-ketosteroids, failure of ACTH to produce a fall in eosinophils, and low or low normal urinary 11-oxycorticoids.

Previous studies from our laboratory have shown that patients with myxedema have an abnormal response to a water load, which is not correctable by cortisone but is corrected by thyroid.

The circulating plasma level of free 17-hydroxycorticoids before and following intravenous administration of ACTH (Jailer method) was used to evaluate adrenocortical function. In this way not only adrenocortical function but also adrenocortical reserve was measured.

Twelve patients with untreated myxedema of 2 to 10 years' duration were studied. The fasting 17-hydroxycorticoid levels were within normal limits in all cases. After ACTH stimulation the increase in plasma 17-hydroxycorticoid levels was normal in 8, slightly below normal in 2, slightly above normal in 1, and definitely above normal in 1 patient (who had coexisting renal insufficiency).

The above determinations were repeated on 2 of these patients after L-triiodothyronine therapy. In 1 patient the plasma 17-hydroxycorticoid values were essentially unchanged from pretreatment values. In the other patient the fasting level was unchanged, but the post ACTH level was lower, being slightly below the normal limit.

The urinary 17-ketosteroid levels were low in all 8 of the patients on whom this determination was made. The 1 patient who had post-therapy ketosteroid studies showed a rise to the low normal range.

These studies thus far indicate that the adrenocortical function and functional reserve of patients with myxedema, as determined by plasma 17-hydroxycorticoid levels before and after ACTH, are within essentially normal limits. The possible effects of hepatic, renal and other factors on these corticoid levels are being investigated.

Influence of Tolbutamide on I^{131} Uptake

By *Emery C. Miller, Jr. and Ernest H. Yount, Jr.*

The mechanism of action of the arylsulfonylureas in lowering blood sugar in normal and diabetic patients remains obscure. Early work with these agents established that depression of thyroid function as measured by I^{131} uptake is a consistent effect of the drugs. This depression of thyroid function, however, is not the mechanism by which hypoglycemia is produced, since lowering of the blood sugar occurs in the absence of significant thyroid depression.

I^{131} uptakes were performed prior to therapy in 18 patients receiving Tolbutamide ("Orinase") in a dosage of 4 Gm./day. Repeat uptake studies were carried out after 5 days on the drug in all patients, and at 2 to 4 week intervals thereafter.

I^{131} uptakes are expressed in terms of uptake rate 10^3K_1/minute rather than 24 hour % since it is our feeling that this method of measurement provides more accurate information. K_1 values tended to decrease in 5 days of treatment but the mean decrease is not statistically significant and the values after therapy are generally within the euthyroid range.

Of particular interest was the finding in 3 patients that a depression of I^{131} uptake into the clearly hypothyroid range was spontaneously corrected with return of K_1 values to normal in 2 or 3 weeks, even though the patient was continued during this time on the same dosage of 4 Gm./day of Orinase.

Depression of thyroid function as measured by I^{131} uptake is a side-effect of Tolbutamide therapy that does not contribute importantly to the hypoglycemic effect of this drug, and is of insufficient degree and persistence to constitute a significant hazard in the clinical management of diabetic patients with Tolbutamide.

Cerebral and Peripheral Utilization of Fructose During Insulin-Induced Hypoglucosemia in Man

By *Holbrooke S. Seltzer, C. Willis Sensenbach and Seymour Eisenberg.* Department of Medicine, University of Texas Southwestern Medical School, and Department of Medicine, V.A. Hospital, Dallas.

Although the brain is supposedly incapable of metabolizing fructose, two sets of observations fostered a study to determine if significant cerebral uptake of fructose occurs during severe hypoglucosemia. 1. We have noted marked amelioration of acute hypoglycemic symptoms in subjects receiving an infusion of fructose throughout the hypoglycemic interval. 2. Slein, Cori and Cori demonstrated noteworthy phosphorylation of fructose by brain hexokinase in the absence of glucose.

Fasting patients who exhibited normal carbohydrate metabolism received fructose intravenously at a constant rate. Cerebral and peripheral arterial-venous differences of glucose and of fructose were determined before and during insulin-induced hypoglucosemia. Cerebral blood flow was also determined before and during the hypoglycemic episode in order to quantitate cerebral utilization of fructose.

Cerebral A-V differences for fructose varied from 1 to −4 mg. % both before and during the hypoglycemic period. At the time of maximal hypoglycemia cerebral blood flows likewise showed no remarkable change from baseline values. Thus, there was no cerebral uptake of fructose despite arterial concentrations of glucose ranging from 14 to 36 mg. %, and despite a sharp decrease in cerebral utilization of glucose. Hypoglycemic symptoms were nevertheless either absent or of only moderate intensity.

Peripheral A-V differences for fructose varied from 9 to 23 mg. % (mean 16 mg. %) before administration of insulin, and from 1 to 28 mg. % (mean 11 mg. %) when hypoglycemia was most profound.

The findings demonstrate that the mitigation of hypoglycemic symptoms afforded by co-existent high blood fructose concentrations is mediated neither by enhanced cerebral metabolism of fructose nor by increased peripheral utilization of fructose, with sparing of circulating glucose for cerebral nutritional demands. The possibility that cerebral metabolism of pyruvate or lactate, blood

concentrations of which increase during fructose infusion, is accelerated during profound hypoglucosemia, is now being investigated.

A New Tool in the Diagnostic Evaluation of Cushing's Syndrome

By *Grant W. Liddle, William S. Coppage, R. Glenn Greene and Donald Island.* Department of Medicine, Vanderbilt University School of Medicine, Nashville.

A fundamental aspect of Cushing's syndrome can now be demonstrated by the use of a standard ACTH *suppression* test. Normal subjects have suppressible 17-hydroxycorticoid-producing mechanisms; patients with Cushing's syndrome do not. The synthetic steroid Δ^1-9α-fluoro-hydrocortisone (ΔFF) is so potent biologically that, normally, oral doses of 0.5 mg. every 6 hours will almost completely suppress ACTH secretion. As a result, corticoid secretion and excretion fall to negligible levels by the second day of suppressive treatment. Of 16 patients with Cushing's syndrome so tested, all have shown complete resistance to suppressive treatment; that is, their supernormal control levels of urinary corticoids have been unaltered by this dose of ΔFF. This has been true regardless of whether the pathologic basis of the Cushing's syndrome was bilateral adrenocortical hyperplasia, benign adrenocortical adenoma, or adrenocortical carcinoma.

Cushing's syndrome may be regarded as a disturbance of homeostasis in which the mechanism for secreting hydrocortisone becomes autonomous, resulting in excessive secretion of this steroid in the absence of any apparent physiologic requirement for it. The nonsuppressibility of the hydrocortisone-secreting mechanism, as demonstrated by the ΔFF test, appears to be a fundamental aspect of Cushing's syndrome; indeed, if the pituitary-adrenal system of these patients *were* suppressible by physiologic amounts of hydrocortisonelike steroids, the disease would never have developed in the first place.

Since all patients with Cushing's syndrome are likely to exhibit supernormal resting corticoid levels and nonsuppressibility of corticoid secretion, the preoperative differentiation of pathologic types must be based on other criteria. In general: 1. Urinary corticoid response to standardized ACTH stimulation is supernormal in bilateral adrenal hyperplasia. 2. Adrenocortical carcinomas are unresponsive to ACTH. 3. Benign adrenocortical adenomas occur very rarely in males and, while producing excessive quantities of 17-hydroxycorticoids, do not usually produce 17-ketosteroids in excess.

Studies on Testicular Interstitial Cell and Adrenal Cortical Response to Adrenocorticotropin and Human Chorionic Gonadotropin in Man.

By *Lester L. Hibbett, Willard R. Starnes and S. Richardson Hill, Jr.* Department of Medicine, the Medical College of Alabama, and the Medical Service, V.A. Hospital, Birmingham.

The present study compares in the same individuals the response of the urinary total 17-hydroxycorticosteroid and 17-ketosteroid levels to the slow intravenous administration of ACTH and human chorionic gonadotropin (HCG). The experiments have been designed to evaluate the effect of HCG on adrenal cortical secretory activity, the possible usefulness of changes in urinary 17-ketosteroid excretion as a rapid test of Leydig cell function, the dose response effect of intravenously administered HCG, the effects of estrogens and androgens and the possible synergistic effect of ACTH and HCG on the excretion of these steroids.

The results indicate that HCG does not lead to increased adrenal cortical secretory activity. The rapid and significant increase in 17-ketosteroid secretion in normal subjects and subjects with panhypopituitarism are in contrast to the lack of such a response in patients with primary hypogonadism and thus point out the usefulness of this procedure in distinguishing primary from secondary hypogonadism and in studying interstitial cell function. There is no advantage to increasing the dose of HCG beyond 10,000 units. Estrogen administration did not change 17-ketosteroid and 17-hydroxycorticosteroid excretion in a patient with primary hypogonadism; similarly, various forms of testosterone did not change 17-hydroxycorticosteroid excretion. There is no synergistic effect between ACTH and HCG.

Nonspecific protein, renal and circulatory effects as a cause of changes in steroid excretion have been excluded and assays were undertaken which have demonstrated no contamination of the HCG preparation with ACTH.

A Simplified Procedure for Studying Fat Absorption and Utilization Using I^{131}-labeled Triolein or Oleic Acid.

By *D. A. Turner.* Department of Surgery, Georgetown University Hospital, Washington, D. C.

Stanley and Thannhauser (1949) reported on the use of I^{131}-labeled olive oil in studies of fat absorption and metabolism. I^{131}-lipid activity of serum was calculated by subtraction of water-soluble I^{131}

activity from total serum activity. The water-soluble I^{131} was separated from lipid-I^{131} by co-precipitation of the serum lipid with serum protein using the Somogyi reagents. Free inorganic I^{131} is also precipitated with serum protein by this procedure and represents an important source of error under certain conditions.

Ruffin et al. and others have measured the total blood or fecal activity after a meal of I^{131}-triolein. No attempt was made to measure actual lipid activity.

A simplified and accurate procedure for the direct estimation of I^{131}-lipid activity in blood or tissue after the oral or intravenous administration of I^{131}-triolein or I^{131}-oleic acid is available.

The method (Turner) has been used in an evaluation of 38 patients with proven pancreatic insufficiency and 26 patients with the malabsorption syndrome without pancreatic insufficiency.

In pancreatic disease triolein absorption is decreased and oleic acid absorption is normal. In mucosal or mechanical abnormalities there is impaired absorption of both the triglyceride and its constituent fatty acid.

The Effect of Unsaturated Fats on Serum Lipids in Normal, Active Subjects

By *William Shapiro, E. Harvey Estes, Jr., and Helen L. Hilderman.* Department of Medicine, Duke University School of Medicine, and V.A. Hospital, Durham, North Carolina.

Commercially available corn oil ("Mazola") was added to the diet of 6 young, normal, active male subjects at various levels of fat and caloric intake. Diets known to be clinically practical and compatible with full activity were utilized for a 5-week period.

Control values were obtained after 1 week of a standard 2700 calorie, 100 Gm. animal fat diet. During the second and third weeks the diet consisted of 2700 calories with 30 Gm. animal fat, 70 Gm. corn oil. In the fourth and fifth weeks the original 2700 calorie, 100 Gm. animal fat diet was reinstituted with the addition of 70 Gm. corn oil supplying an additional 630 calories. Corn oil was taken throughout the day as an emulsion in fruit juice. Sera were analyzed weekly. Body weights did not change significantly.

The most striking effects were on free and total cholesterol. Mean values dropped from 67.9 mg.% and 218 mg.% to 30.2 ($p < .01$) and 158 ($p < .01$) respectively, by the end of the third week. They remained depressed, though less so at the end of the fifth week ($p < .01$, $p < .05$ respectively). Total lipid dropped more slowly, reaching a nadir at the end of week four ($p < .01$), rising at week five ($p < .2$). The lipid phosphorus followed the pattern of total lipid, but the drop was less striking, the only significant difference being at week four ($p < .05$). Lipid iodine number rose significantly, beta lipoprotein percentage dropped slightly during corn oil feedings. These data confirm the reports of others concerning the effect of unsaturated fats on serum lipids. These effects were obtained in active individuals, utilizing a readily available and inexpensive material added to diets practical for long term use. Corn oil was shown to be more effective when combined with restriction of animal fat intake.

Biochemical Changes Associated with the Intravenous Administration of Ammonium Salts in Normal Subjects

By *Malcolm P. Tyor and William P. Wilson.* Departments of Medicine and Psychiatry, Duke University School of Medicine and V.A. Hospital, Durham, North Carolina.

A positive peripheral arterial-venous difference of ammonia has been observed in certain patients with cirrhosis. The biochemical alterations which may accompany this "binding" of ammonia by cells are poorly understood.

Nine patients, without clinical or laboratory evidence of liver disease, received intravenous infusions of 42 to 70 mEq. of NH_4^+ as 0.155 M ammonium chloride (3 patients) or 0.155 M ammonium lactate (6 patients). Patients were infused continuously over a one-hour period. Brachial arterial samples were taken before infusion, at 30 and 60 minutes after initiation of infusion, and after discontinuation. They were analyzed for ammonia, urea, glucose, potassium, sodium, pH and CO_2 combining capacity. Simultaneous venous samples were obtained from the same arm and ammonia concentration measured. The data were analyzed by comparing the differences between the means of paired values.

The following changes were considered most significant: 1. A progressive increase in arterial ammonia concentration was observed at 30 minutes (range 0.13 to 0.38 mEq./L.) and 60 minutes (range 0.26 to 0.51 mEq./L.), with rapid return to control levels (range 0.02 to 0.05 mEq./L.). 2. Arterial-venous ammonia differences increased markedly at 30 minutes, when compared with control levels ($d = +0.13$ mEq./L., $p = < 0.01$), indicating the passage of ammonia into the periphery. Sixty-minute values showed no further change ($p = > 0.50$), despite the progressive increases of arterial ammonia

concentration during this interval ($p = < 0.01$). A subsequent decline of arterial-venous differences to control levels ($\bar{x} = 0$) was observed. 3. Significant increases in potassium concentration were present at 30 minutes, when compared with control values ($d = +0.39$ mEq./L., $p = < 0.01$). No further change was observed at 60 minutes ($p = > 0.50$); however, potassium concentrations fell significantly during the postinfusion period ($d = -0.24$, $p = < 0.02$). These differences in potassium paralleled arterial-venous ammonia differences and could not be accounted for by changes in pH and CO_2 combining capacity.

Body Fluid Metabolism. II. The Relative Efficacy in Man of $NaHCO_3$ and Sodium Lactate as Alkalizing Compounds, and of NH_4Cl as Acidifying Compounds

By *James D. Hardy, Don M. Turner and Virginia Ward.* Department of Surgery, University of Mississippi Medical Center, Jackson.

Critically ill patients, perhaps with renal or hepatic decompensation or with diabetes mellitus, often require corrective therapy for deranged acid-base balance. In the past, alkalinization has been most generally achieved with sodium lactate and acidification with ammonium chloride. However, theoretically, $NaHCO_3$ should represent a more efficient alkalinizing compound because its anion (HCO_3) is rapidly excreted and does not need to be metabolized, as does lactic acid. Similarly, the ammonium ion liberated upon infusion of NH_4Cl could conceivably produce toxic effects in patients with borderline liver failure.

Twenty subjects in normal acid-base balance and without hepatic or renal disease were divided into four groups of five each. After control studies of blood pH, pCO_2, Na, K, Cl, and CO_2 combining power had been done, 1 L. of M/6 solution each of $NaHCO_3$, NH_4Cl, and sodium lactate, and of M/6 HCl was infused over a period of 1½ hours. Further measurements of the above levels were continued during the infusion and afterwards.

Similar alterations in blood pH were produced by $NaHCO_3$ and sodium lactate; similarly, NH_4Cl and HCl. Hydrochloric acid and $NaHCO_3$ are satisfactory agents for clinical use. Thus far, our data fail to confirm published reports that induced alkalosis produces a fall, and acidosis a rise, in the plasma potassium level. (Army Contract No. DA-49-007-MD-627).

On the Mechanism of the "Shunt" Pathway of Uric Acid Synthesis

By *James B. Wyngaarden, Alberta Blair and Janet Schuck.* National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Department of Health, Education and Welfare, Bethesda, Maryland.

To explain the prompt and excessive conversion of glycine-N^{15} into urinary urate in certain gouty subjects, Stetten postulated a "shunt" pathway whereby glycine was converted to uric acid without the intervention of nucleic acid purines as intermediates. In the present study evidence was secured for the existence of such a shunt pathway in normal, gouty, and leukemic subjects who were given orally 5–20 μc. of glycine-1-C^{14} to label urinary purines. Purine bases were isolated from daily urine samples by combination of copper precipitation and Dowex-50-H^+ resin column technics. Several purines were eventually secured as crystalline products. In control and gouty subjects, adenine, hypoxanthine, 7-methyl guanine and guanine were maximally labeled on the first day and then declined to rather constant low isotope levels. In the leukemic subject there was a secondary rise in isotope concentrations, with new maxima on the 7th to 12th days. These patterns suggested that early cleavage of newly synthesized nucleotides, with subsequent oxidation of purine bases to uric acid, occurred in all subjects, and accounted for the prompt appearance of isotope in uric acid. Whether the abnormal shunt pathway believed present in certain gouty subjects involves an accentuation of one of these cleavage pathways or some other defect remains to be determined. In addition, the conversion of labeled nucleotides to nucleic acids, and their subsequent catabolism, probably accounts for the continued low-level enrichment of purines in the control and gouty subjects, and for the late accentuation of labeling of urinary purine bases in the leukemic subject. From the observation that urinary xanthine is never as highly labeled as uric acid it is suggested that there are distinct nucleotide pools of differing metabolic activity and that at least one of these contributes significant quantities of poorly labeled purine bases to the urine. An alternative pathway of urate synthesis not involving free xanthine as an intermediate would also explain this finding.

Hypocalcemia in Acute Barbiturate Intoxication

By *John J. Canary, Laurence H. Kyle, Leonard B. Berman and George E. Schreiner.* Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Seven patients without skeletal or gastrointestinal disease, hospitalized for acute barbiturate intoxication were found to be hypocalcemic. The mean serum calcium level was 8.2 mg./100 ml. and the range, 7.5 to 8.8 mg. In this group, three patients with the most profound hypocalcemia had associated hypophosphatemia; the mean serum phosphorus level was 1.7 mg./100 ml., range 1.1 to 2.2 mg. None had overt or latent tetany. Blood calcium and phosphorus values slowly returned to normal levels with or without therapeutic hemodialysis.

During dialysis conducted on three patients, serum calcium was restored to normal in two instances and to a hypercalcemic level in the third by the addition of calcium to the bath. Within hours following dialysis, serum calcium concentrations fell to or below the predialysis levels, subsequently returning to normal.

These abnormalities have been found in patients intoxicated with amobarbital, pentobarbital, and phenobarbital. Two other patients who ingested secobarbital had low blood barbiturate levels and normal serum calcium and phosphorus concentrations. Correlation between barbiturate level and depression of serum calcium and phosphorus concentrations could be made only in the two instances of phenobarbital intoxication. No increased urinary excretion of calcium or phosphorus was demonstrable in any of the patients. The addition of calculated amounts of several barbiturates to normal serum, resulting in blood levels corresponding to those found in the patients studied, produced no change in calcium or phosphorus levels.

Two possible explanations for this hitherto unrecognized variety of hypocalcemia appear reasonable. First, calcium with or without phosphorus may be bound to barbiturate in the gastrointestinal tract. Second, a complex between barbiturate and calcium with or without phosphorus may be formed which is sequestered in some extravascular space.

A Thyroid-Stimulating Hormone Assay Based on I^{131} on Rana Catesbeiana Tadpoles

By *C. Y. Bowers and Albert Segaloff.* Alton Ochsner Medical Foundation and the Department of Medicine, Tulane University, New Orleans.

The only assay sufficiently sensitive to measure human serum thyroid-stimulating hormone levels is that of D'Angelo and Gordon. This assay employs a measurement of the thyroid cell height in starved *Rana pipiens* tadpoles. Since the tadpole seems to be most sensitive to TSH, we have employed starved *Rana catesbeiana* tadpoles using radioactive iodine uptake as the end point. The tadpoles are brought directly from the field, weighing 2–4 Gm., and with hind legs less than 5 mm. in length. After 7 to 14 days of starvation in $\frac{1}{10}$ amphibian Ringers solution, they are injected intraperitoneally with a single dose of TSH dissolved in 0.05 cc. distilled water and sacrificed 72 hours later. Twenty-four hours before sacrifice they are injected intraperitoneally with one microcurie of I^{131} in 0.05 cc. The thyroid gland is dissected out under a microscope and the radioactivity counted. The counts in these stimulated thyroid glands are compared to those of control thyroid glands of animals treated the same except for the administration of TSH. Under these conditions we have been able to show a definite effect from 5 milliunits of the USP TSH reference standard.

GASTROINTESTINAL SYSTEM

The Deglutition Pressure Gradient in Normal Subjects and Patients with Dysphagia

By *E. C. Texter, Jr., H. W. Smith and J. H. Stickley.* Gastroenterology Laboratory and the V.A. Research Hospital, Northwestern University Medical Center, Chicago. (Aided by a grant from the U. S. P. H. S.)

The dynamics of deglutition were studied employing simultaneous registration of intraluminal pressures from three points, 5 cm. apart, concurrently with fluoroscopic study of esophageal transport. Forty-three studies were carried out in 20 normal subjects and 19 patients with dysphagia. An adaptation of the open-tipped catheter technic of Quigley was used, the most distal tube being placed immediately above the diaphragm. The duration and amplitude of the deglutition complexes were measured following the barium swallows and dry swallows.

The parameters of the complexes from the normal subjects were similar to those reported by others. The duration of the complex increased in the terminal esophagus and "ampullary" type complexes were observed. The maximum pressure was recorded from the distal esophagus, 5 cm. above the level of the diaphragm.

Abnormalities in the deglutition pressure gradient were observed in all of the subjects with dysphagia. Dysphagia secondary to achalasia was characterized by the presence of nonpropulsive waves of low pressure and prolonged amplitude at all levels of the esophagus studied. Both the duration and amplitude of the deglutition pressure waves and achalasia differed significantly from the normal ($p < 0.01$). The patients with symptomatic hiatal hernia had normal deglutition complexes except at the terminal segment of the esophagus or supradiaphragmatic segment of the stomach where the duration of the complex was prolonged and the amplitude reduced. The parameters differed significantly from the normal but did not differ significantly from the achalasia group. Absence of the deglutition complex in the terminal esophagus was observed in one patient with an esophageal ulcer. A decrease in amplitude and prolongation of the deglutition complex was also observed in the terminal esophagus in patients with functional dysphagia.

The pressure changes within the lumen of the digestive tract are correlated with propulsive activity. The demonstration of the common defect related to esophageal transport—that of abnormally low and prolonged intraluminal pressure waves in the terminal esophagus in a wide variety of disorders—strongly suggests that the dysphagia common to these disorders has a common physiologic basis in disturbed motor function.

Pepsin in Gastric Physiology. Effect of Ulcer Diathesis, ACTH, Histamine, Anesthesia-Operation, Banthine, Pilocarpine, Epinephrine and Sedation Upon Blood and Urine Pepsin Levels and Upon Gastric Acidity

By *Albert L. Meena, J. Harold Conn, Hans Naumann and James D. Hardy.* Departments of Surgery and Pathology, University Medical Center and the V.A. Hospital, Jackson, Mississippi.

There is renewed interest in the relation between pepsin production, gastric acidity, and peptic ulcer. Most reported studies have dwelt upon pepsin concentrations in gastric juice and urine, but recently it has become possible to measure pepsin levels in blood. Among the more outstanding studies have been those of Bucher, of Mirsky and of Gray et al.

One purpose of the present study was to confirm and to extend the information available regarding the acid-pepsin factor and its role in duodenal ulcer production. A second purpose was to examine the effects of cortisone, histamine, anesthesia-operation, banthine, pilocarpine, epinephrine, and sedation (Thorazine and Serpasil) upon pepsin production. The methods used for blood and uropepsin determination were patterned after the method of Anson and Mirsky.

One hundred and ten subjects have been studied measuring the uropepsin and blood pepsin levels under the above conditions. Included in the 110 and 25 patients who also had a 24-hour gastric analysis.

A wide range of uropepsin and plasma pepsinogen values in normal subjects was found. Moreover, in a given individual there was a considerable fluctuation from day to day. Correlation between uropepsin and plasma pepsinogen values and the gastric acidity was poor. The results in ulcer patients were not constant, although high values were found in peptic ulcer with obstruction or bleeding and low values after gastrectomy. Following surgical trauma there were rather wide fluctuations of values with a tendency toward a decrease in pepsinogen production on successive postoperative days. Some drugs caused a marked effect, partly stimulating, partly inhibiting, but here again no uniformity was observed.

The Effects of ACTH and Adrenal Steroid Hormones on Gastric Secretion

By *David A. Dreiling and Henry D. Janowitz.* Department of Surgery and Gastroenterology Clinic, The Mount Sinai Hospital, New York.

A relationship between gastric secretion and the adrenal cortex has been suggested from clinical and experimental data. The clinical evidence is the appearance of peptic ulceration and the worsening of ulcer symptomatology during states of induced hypercorticalism. The experimental evidence includes the generally accepted observation of a direct correlation between the blood pepsinogen and urinary pepsin with the level of adrenal cortical activity. In addition, Gray has presented investigative data suggesting a direct stimulatory action of the adrenal hormones on gastric acid secretion. Controversy still exists regarding this phenomenon.

The present study was undertaken to determine the secretory effect of the adrenal hormones on human gastric secretion. The volume flow, free and total acidity, and pepsin secretion were determined in specimens obtained with the continuous suction technic at hourly intervals before and for 6 hours after intravenous injection of ACTH (25–40 mg.), hydrocortisone (25–85 mg.), and metacortelone (50 mg.). The investigative series included 37 subjects without gastrointestinal disease, 14 patients with duodenal ulcer, and 4 patients with gastric ulcers. The data obtained in these studies indicated that there was no stimulatory effect by these hormones on the volume rate of gastric secretion, the acid output, and the enzyme elaboration. The findings suggest that the adrenocortical hormones are not direct gastric secretagogues in the usual sense. Therefore, the mechanism of peptic ulceration during adrenocortical hormone therapy remains obscure, unexplained, and cannot be ascribed to a secretagogue action.

Urinary Excretion of 17-Hydroxycorticosteroids in Peptic Ulcer Disease

By *Alvin J. Cummins, Michael L. Gompertz and Marie H. Jones.* Gastrointestinal Laboratory, Department of Medicine, University of Tennessee Medical Units, and the Gastrointestinal Section of the Medical Service, V.A. Medical Teaching Group (Kennedy) Hospital, Memphis.

Despite considerable clinical and laboratory investigation, the relation of adrenocortical function to the pathogenesis and course of human peptic ulcer disease is uncertain. The present study was designed to determine whether evidence of excess adrenocortical activity could be demonstrated in a group of ulcer patients by measurement of urinary excretion of 17-hydroxycorticosteroids.

Steroid assays were made on 24-hour urine specimens collected from 20 radiographically proven active duodenal ulcer patients, 9 active gastric ulcer patients, and 26 nonulcer control patients. Basal steroid excretion was calculated as the mean of two 24-hour collections. In 10 duodenal ulcer subjects, 7 gastric ulcer cases and 11 controls, a third 24-hour urine specimen was studied after the administration of two injections of 40 C. U. of ACTH gel at twelve-hour intervals, to assess adrenocortical responsiveness. In most cases, to correct possible collection timing errors, urinary excretion of creatinine was measured and 17-hydroxycorticosteroid/creatinine ratios calculated.

Basal 24-hour steroid excretion for the control subjects was 7.3 mg. (S.E. = 0.68), for the duodenal ulcer patients 6.9 mg. (S.E. = 0.89), and for the gastric ulcer cases 5.7 mg. (S.E. = 1.05). Statistical analysis reveals no significant difference between the means for either ulcer group and the controls. Following ACTH administration the steroid excretion for the controls rose 351%, for the duodenal ulcer patients 349%, and for the gastric ulcer cases 336%. Again, there is no significant difference here or in the urinary steroid/creatinine ratios for the three groups.

It is concluded that by measurement of urinary excretion of 17-hydroxycorticosteroids no quantitative alteration in adrenocortical function can be demonstrated in the patient with active peptic ulcer. Adrenal responsiveness to the type of stimulus used likewise indicates no detectable aberration. Admittedly failing to provide a conclusive answer, this study offers no support to the concept of adrenal hypercorticism in the pathogenesis of peptic ulcer.

Study of Hepatic Cell Integrity in the Differential Diagnosis of Icteric Patients with Lymphomatous Disease

By *David W. Molander and John S. LaDue.* New York, N. Y.

In a group of patients with malignant lymphoma (including Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma), the serum glutamic oxalacetic transaminase (SGO-T) activity was measured in order to determine the presence of hepatic parenchymal cell necrosis in association with liver involvement in these disorders.

The SGO-T activity was within normal limits in the vast majority of lymphomatous patients with enlarged livers. In patients with lymphoma, increased SGO-T activity was found to be the most useful laboratory test to determine acute hepatic parenchymal cell damage generally due to viral hepatitis. The SGO-T activity was either normal or only slightly increased in instances of jaundice due to intrahepatic infiltration by lymphoma, extrahepatic obstruction by tumor or in instances of hemolytic anemia.

Radiation therapy to the liver or chemotherapy (nitrogen mustard) did not alter the SGO-T activity.

In general, lymphomatous infiltration of the liver is not accompanied by significant hepatic cell necrosis. Histologic study of livers of several of the patients at autopsy confirmed this.

A Comparison of Bromsulphalein and Radio-Colloid Removal Rates for Determining Hepatic Blood Flow

By *Cheves McC. Smythe*. Medical College of South Carolina, Charleston.

Hepatic blood flow (EHBF) determined by bromsulphalein (BSP) removal has been compared with flow obtained simultaneously with the radioactive colloidal chromic phosphate (RCCP) disappearance method of Dobson in 14 dogs anesthetized with pentobarbital. The ratio of flow determined by RCCP disappearance to that determined by BSP removal averaged 1.11 (S.D. ± 0.2). The range was 1.32 to 0.64, but in only four instances did the ratio diverge more than 0.16 from unity. Mean flow by both methods was 42 ml./Kg./min.

Thirteen of these dogs were suddenly bled an average of 18 ml./Kg. (range 15 to 21 ml./Kg.) by syringe, and hepatic blood flow redetermined by RCCP removal. Compared with control flows estimated from both BSP and RCCP removal rates, there was no significant decrease in flow. (Control 42.8 ml./Kg./min. Post bleeding 41.3 ml./Kg./min.) This is in marked contrast to other studies. The constancy of RCCP removal rates in the face of challenging hemorrhage leads one to surmise that RCCP disappearance is dependent upon variables other than hepatic blood flow.

In a group of miscellaneous patients (congestive heart failure, cirrhosis of the liver, sickle cell disease) in whom data collected by the BSP method indicates that hepatic blood flow is reduced, delayed disappearance times for RCCP were not found. Three possible explanations of this divergence from expected hemodynamic patterns may be postulated: 1. The avidity of the littoral cells for the material may be such that removal, although largely dependent upon flow, may vary independently. 2. The RCCP disappearance rate may be an index of phagocytic efficiency and its close agreement with figures obtained by BSP removal only incidental. 3. In the presence of hepatic disease, anoxia, etc., the efficiency of removal may be so decreased that material escapes into the hepatic vein, leading to overestimation of hepatic blood flow.

Vitamin B_{12} Metabolism in Liver Disease

By *Thomas D. Stevenson*. Institute of Medical Research, University of Louisville, Louisville, Kentucky.

The serum vitamin B_{12} level, determined by microbiologic assay utilizing *Euglena gracilis* as test organism, is elevated above the normal range in patients with liver disease. The mechanism for this increase in the vitamin B_{12} level in patients with liver disease is not known.

The absorption of radioactive vitamin B_{12} has been studied in patients with liver disease, utilizing fecal and urinary excretion as an index of absorption. The absorption of the vitamin is within the normal range and there is no evidence from these studies that absorption is increased or influenced by the increased serum level of vitamin B_{12}.

The urinary excretion of vitamin B_{12} determined by microbiologic assay is extremely variable and erratic. No consistent evidence of an increased urinary excretion of vitamin B_{12} has been found in patients with liver disease, and this technic appears to be unreliable as an index of excretion.

In contrast to the abnormal plasma clearance of radioactive vitamin B_{12} found in patients with myeloid leukemia with elevated vitamin B_{12} levels, the plasma clearance of intravenously administered radioactive vitamin B_{12} is normal in patients with liver disease. The effect of hypoproteinemia and renal insufficiency on the plasma clearance of vitamin B_{12} has also been investigated. The vitamin B_{12} content of the cirrhotic liver is decreased as determined by microbiologic assay.

The effect of folic acid and methionine on the serum vitamin B_{12} level has also been studied in patients with liver disease. Methionine, when given intravenously to patients with liver disease, has produced coma with no alteration in vitamin B_{12} level. In spontaneous hepatic coma, however, the vitamin B_{12} level is greatly increased.

The data indicate that there is no alteration in the absorption of vitamin B_{12} in patients with liver disease with elevated serum levels. The normal plasma clearance of radioactive vitamin B_{12} demonstrates a fundamental difference between the elevated vitamin B_{12} level found in liver disease and myeloid leukemia. Release of the vitamin from hepatic cells is the most likely explanation for the increased serum level.

The Influence of Pancreatic Lipase on Fat Absorption. The Behavior of Serum Lipids, Lipoproteins and Serum Turbidity

By *Helmut Redetzki*. Department of Medicine, University of Texas and University of Hamburg School of Medicine.

Repeated fat loading (1 Gm. olive oil per Kg. by duodenal intubation) with continuous analysis of serum lipids revealed a constant type of fat absorption in the same individual. The use of lipase caused

differences in the composition and behavior of blood lipids.

Following a fat load, the specific type of response was first determined by estimating total lipids, cholesterol, phospholipids, lipoproteins and serum turbidity in regular intervals. Four days later an identical experiment was made, however, with the addition of lipase to the olive oil. Forty-six adult persons were used for the experiments.

The following effects of lipase could be observed: 1. Lipase accelerated the absorption of triglycerides. 2. Lipase application diminished the serum turbidity as revealed by nephelometry and measurement of density. 3. Lipase favored the binding between proteins and lipids.

Paper electrophoresis of lipoproteins showed an absolute increase of the β-lipoprotein fraction and a relative increase to the nonprotein bound lipids.

KIDNEY

The Effect of Arterial Pressure Alterations on The Renal Red Cell Shunting Mechanism

By *Lawrence S. Lilienfield and John C. Rose.* Cardiovascular Research Laboratory, Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Pappenheimer and Kinter have described a renal red cell shunting mechanism which, they suggest, regulates renal resistance and which receives the energy for its function from the renal arterial pressure. These observations have great significance, for they imply that intrarenal red cell concentration rather than arteriolar tone controls renal resistance.

Application of a single circulation indicator-dilution technic in 16 dogs has confirmed the existence of the shunt. To study the effects of pressure alterations on this mechanism, the dynamic intrarenal hematocrit in a kidney of anesthetized dogs has been measured under control (normal) blood pressure conditions and again following reduction in arterial blood pressure induced by removal of blood from the circulation. A mixture of I^{131} albumin and Cr^{51}-tagged red cells was injected rapidly into the renal artery, and total renal blood flow was collected for at least one minute at two-second intervals from an artificially lengthened exteriorized renal vein. Arterial pressure was continually monitored. The aortic pressure was then reduced at least 50% and the experiment repeated. From a plot of time vs. radioactivity of the supernatant plasma and the washed red cells of each sample, the mean circulation times of the red cells and plasma were calculated. From this data and the measured renal blood flow, the intravascular red cell and plasma volumes were determined. The calculated intrarenal hematocrit was compared with the hematocrit of the venous blood (Wintrobe).

The ratio of intrarenal to large vessel hematocrit averaged 0.89 in the control group and 0.90 in the hypotensive group, despite an average four-fold increase in renal vascular resistance. These results indicate that under the conditions described, renal resistance is not controlled by intrarenal red cell concentration, and the red cell shunt mechanism is not pressure dependent.

Relation of Serum Bicarbonate to Bicarbonate Diuresis During Diamox Administration

By *Richard M. Portwood, Floyd C. Rector, Jr., Robert Cade and Donald W. Seldin.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

Refractoriness to Diamox during metabolic acidosis has been attributed to depression of serum bicarbonate, thereby reducing the quantity filtered so that the uncatalyzed hydration of CO_2 may furnish sufficient H^+ ions to effect complete HCO_3^- reabsorption despite carbonic anhydrase inhibition. This hypothesis was tested in normal subjects in whom the serum (HCO_3^-) was reduced to 14–17 mEq./L. by hyperventilation during maximum water diuresis.

During hyperventilation alone HCO_3^- and K^+ excretion increased from 16 $\pm$ 5 to 27 $\pm$ 15 ($p < .10$) and 33 $\pm$ 8 to 55 $\pm$ 15 ($p < .02$) μEq./min., respectively. Urine pCO_2 decreased from 46 $\pm$ 6 to 30 $\pm$ 7 mm. Hg ($p < .01$).

Diamox administered during hyperventilation increased HCO_3^- excretion to 544 $\pm$ 150 μEq. min. despite reduction in serum (HCO_3^-) to 14–16 mEq./L. and a marked decrease in filtered HCO_3^-. It is noteworthy that urine pCO_2 remained virtually unchanged (47 $\pm$ 8 to 50 $\pm$ mm.Hg, $p > .3$).

Diamox administered alone in the presence of normal serum (HCO_3^-) produced no greater HCO_3^- diuresis than Diamox + hyperventilation, but urine pCO_2 rose strikingly from 47 $\pm$ 8 to 102 $\pm$ 27 ($p < .01$).

Because K^+ excretion rises and urine pCO_2 falls, it is suggested that the small HCO_3^- diuresis during respiratory alkalosis results from decreased distal tubular H^+ secretion. The marked HCO_3^- diuresis with no rise in pCO_2 obtained with Diamox + hyperventilation suggests proximal inhibition of HCO_3^- reabsorption by Diamox linked with diminished distal tubular H^+ secretion induced by respiratory alkalosis.

These data suggest that the magnitude of distal tubular H^+ secretion, rather than the filtered HCO_3^- load, governs the response to Diamox. Depressed serum (HCO_3^-) during metabolic acidosis is associated with Diamox refractoriness because of increased distal H^+ secretion. Comparable depression of (HCO_3^-) during respiratory alkalosis is associated with HCO_3^- diuresis following Diamox because of depressed distal tubular H^+ secretion.

The Effect of Dilution and Dehydration in Patients with Edema and Hyponatremia

By *Mackenzie Walser and Jack Orloff*. Laboratory of Kidney and Electrolyte Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland.

Normally, the rate of excretion of solute-free water during suppression of antidiuretic hormone (ADH) secretion is at least 4% of the filtration rate, even in edematous subjects. Consequently, persistent hyponatremia in association with reduced osmolality of body fluids must be indicative of impaired water diuresis. This might be related (1) to continued secretion of ADH despite hypotonicity or (2) to the operation of an intrinsic renal concentrating mechanism in the absence of ADH. The latter has been demonstrated to cause impaired water diuresis when filtration rate is reduced, provided that sodium excretion is negligible (Berliner and Davidson).

In an attempt to distinguish between these possibilities water loads were administered to 5 patients with hyponatremia, edema, diminished filtration rate and hypertonic urine. Three patients had carcinomatosis, 1 heart failure, and 1 glomerulonephritis. The diuretic response expressed in relation to filtered loads of water and solute was normal in 2, minimal in 2, and absent in 1. Thus, despite severe hypotonicity, 4 patients formed a dilute urine following water administration.

Correction of hyponatremia was achieved by water restriction in 2 patients, by oral loads of urea in addition in 2, and by the administration of 15% mannitol in 1. Balance data obtained during correction of hyponatremia in 2 patients indicate that the rise in plasma osmolality was reflected throughout a volume approximating total body water. No evidence for osmotic reactivation of cell constituents was found.

Although these observations do not permit a definite choice between the two alternative mechanisms of hyponatremia, it is unlikely that a rise in filtration rate alone could account for the water diuresis. Furthermore, sodium excretion was appreciable in 2 patients. The data favor the view that potentially reversible ADH secretion may be responsible for the persistent hyponatremia.

MC-9367 (Squibb N-[5 Sulfamoyl-1,3,4-Thiadiazol-2-y 1] Propionamide), Carbonic Anhydrase Inhibitor, as a Diuretic

By *Arthur Ruskin and Belle Ruskin*. Department of Medicine, University of Texas Medical Branch, Galveston.

This propionylamino derivative of acetazoleamide in preliminary studies (1954) showed diuretic effects comparable to and, in some instances, superior to, acetazoleamide (Diamox).

In 10 edematous (9 in heart failure) human subjects 0.5 Gm. MC-9367 was given intravenously. First and second hour and twenty-four hour urinary volumes, sodium, potassium and chloride were compared with controls (24- and one-hour). The hourly specimens were drawn by catheter with hydration. Mercaptomerin 1 cc. I.V. 24-hour excretions were also measured in the same patients for further comparative diuretic evaluation.

Twenty-four hour diureses of 25 to 300% over the controls resulted in 7 out of 10 cases. Sodium excretion rose in 9 out of 10 cases from 65 to 1730%, potassium in 8 out of 10 cases from 30 to 250%, chloride in 5 out of 10 cases from 30 to 190%. The increases in electrolyte and water excretion occurred the first hour after administration of the drug and were frequently maximum the first or the second hour. Superior diuretic effects (130 to 210% of the MC-9367) from Mercaptomerin were seen in 5 cases, inferior or equal (55 to 100% of the MC-9367) in the other 5 instances. Toxic effects, lasting up to 24 hours, included parethesias and, in 2 patients, psychoses, both in old patients with good diureses.

Oral MC-9367 in 9 edematous (8 in heart failure) patients in daily doses of 0.5, 0.75, 1.0 Gm. resulted in increased urinary excretion in 8 of 9 cases of 30 to 700% over the control days before and after the drug administration. Natriuresis generally paralleled diuresis, which often lasted 2 or more days. The 3 dosages were equivalent in effect. One of the 9 patients developed drowsiness, another a

confused state for 24 hours, which did not recur upon readministration of the drug. No deleterious effects upon renal function noted.

Conclusion: MC-9367 is a moderate oral diuretic, harmless in short courses but with some alarming neuropsychiatric effects on intravenous administration.

A Comparison of the Nephrotoxic Effects of Sodium Acetrizoate (Urokon) When Injected into The Aorta in States of Relative Hyperhydration and Dehydration

By *George C. Morris, Jr.* Cora and Webb Mading Department of Surgery, and the Department of Pharmacology, Baylor University College of Medicine, Houston. (Aided by a grant from the U.S. P.H.S. and the Houston Heart Association.)

Renal insufficiency following translumbar aortography has been encountered with increasing frequency during recent years. The nephrotoxic propensity of sodium acetrizoate (Urokon), when injected into the aorta above the level of the renal arteries, has been demonstrated in the dog. In view of previous experimental work showing the importance of dehydration in the development of renal insufficiency associated with hemoglobinuria, and during treatment with certain sulfa derivatives, it was felt that the magnitude of the renal injury resulting from nephrotoxic doses of this drug might vary with the urinary output at the time of injection.

With this background, two groups of dogs were studied to determine the effects of hyperhydration and relative dehydration at the time of intra-aortic injection of nephrotoxic doses of 70% sodium acetrizoate (Urokon). Renal function was determined using creatinine and PAH clearance studies in 24 dogs before, and three to five days after, the rapid intra-aortic injection of .6 cc./Kg. of 70% sodium acetrizoate (Urokon). Twelve dogs were allowed unrestricted fluid intake prior to injection and then given 40 cc./Kg. of 5% glucose and water intravenously immediately prior to injection. The other 12 dogs were denied fluid intake for a period of 24 to 36 hours prior to injection. Follow-up determinations of renal function showed a 70% reduction in glomerular filtration rate and renal blood flow in the dehydrated group, compared to only 23% in the hyperhydrated group. This study suggests that adequate hydration may reduce the nephrotoxic potentialities of sodium acetrizoate (Urokon) as used in clinical aortography.

Membranous Glomerulonephritis

By *Alvin E. Parrish and John S. Howe.* V.A. Hospital, and the George Washington University, Washington, D. C.

Nine patients presenting with the clinical syndrome of nephrosis were found to have membranous glomerulonephritis and have been followed with renal biopsy, renal function, and careful examination of their urinary sediment. In five patients with the histologic lesions of acute membranous glomerulonephritis, serial biopsies have been obtained. In two of these there was a regression of the acute lesion but the histology did not return to normal even after one year, despite lack of clinical findings, abnormal urinary sediment or marked renal functional impairment. In two there was a progression of the lesion to lobular glomerulonephritis. One patient initially showed a lesion resembling acute proliferative glomerulonephritis.

In four other patients the histologic picture was that of chronic membranous glomerulonephritis. This resembled the biopsies of the "acute" lesion two months or more from the onset. In these patients symptoms suggesting the nephrotic syndrome had been present for 2 to 8 years. Renal function was slightly to moderately impaired.

In all patients there was hypoproteinemia, proteinuria, hypercholesterolemia, edema, and fat in the urinary sediment.

It is postulated that membranous glomerulonephritis is a common cause of nephrosis and should be considered in any patient without a previous history of renal disease who develops the nephrotic syndrome.

Type-Specific Antibody Response to M Protein of Nephritogenic Streptococci in Glomerulonephritis

By *Mary Alice Bone, A. I. Braude and Herman Kleinman.* Department of Internal Medicine of the University of Texas Southwestern Medical School, Dallas; and the Red Lake Indian Reservation, Red Lake, Minnesota.

The predominant isolation of type 12 streptococci from patients with acute glomerulonephritis led to the hypothesis that only certain streptococcal types have nephritogenic properties. If this hypothesis is valid, patients with glomerulonephritis should develop type-specific antibodies to streptococcal M protein of nephritogenic types. Studies were therefore undertaken (1) to detect such antibodies, the studies to be used as immunologic

evidence supporting the thesis that nephritogenic streptococci exist; and (2) to provide a serologic method for investigating the role of nephritogenic streptococci in numerous nephritics from whom nontypable or no streptococci were isolated.

For this purpose a complement fixation test was devised using as antigens M proteins obtained by acid extraction from a type 12 streptococcus, and from a Red Lake type of group A streptococcus isolated from a nephritic patient in Dallas. Antibodies were sought in sera collected from patients two years after their recovery from acute epidemic glomerulonephritis on the Red Lake Indian Reservation in Minnesota. Because residents of this isolated community had experienced no streptococcal disease during the five years preceding their nephritis, they were excellent subjects for controlled study.

Among sera from 54 patients, 53 fixed complement with Red Lake extract and 23 did so with type 12 extract. Sixteen of the sera positive with both extracts were then absorbed with an indifferent streptococcal type. Upon retesting, 14 remained positive with the Red Lake antigen, whereas none was positive with the type 12 antigen. The only serum nonreactive with Red Lake extract before absorption was obtained from a patient having three recurrent episodes of glomerulonephritis.

These results indicate that the Red Lake streptococcus is a separate nephritogenic type, immunologically different from type 12; is widely distributed geographically; and evokes a prolonged immunologic response which provides evidence by complement fixation of past nephritogenic infection.

Evaluation of the Usefulness of "Sterile-Voided" Urine Cultures in the Female; a Comparative Study of 100 Hospitalized Female Patients

By *A. Donald Merritt and Jay P. Sanford.* Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

Urinary tract infections remain among the most common diseases in which antibacterial therapy is useful. However, only a portion are diagnosed by clinical features. Catheterized urine culture, the most useful technic for detecting infection, is not without risk. Therefore, the feasibility of using "sterile-voided" rather than catheterized urines for culture was evaluated in 100 consecutive female admissions to a general medical ward.

Nurses were requested to prepare patients for routine catheterization, but instead of inserting a catheter, a mid-stream voided specimen was collected. Aliquants of urine, diluted 10^{-1} and 10^{-3} were incorporated into "pour plates" for bacterial counts. The remaining urine was cultured. Patients with "sterile-voided" urines containing > 5,000 organisms/ml. were catheterized and studies were repeated. One of us obtained a history which included: age, race, dysuria, frequency, nocturia, incontinence, urinary calculi, genitourinary infections, pelvic operations, pregnancies, previous catheterizations, diabetes mellitus and examined for flank and suprapubic tenderness. Correlation with admission diagnoses, fever, leukocyte count, urinalysis, NPN and PSP excretion was made.

The over-all incidence of significant bacteruria was 12%. In all but 4% of the patients, the catheterized urine correlated with the "sterile-voided" urine. "Sterile-voided" urines contained < 5,000 organisms/ml. in 74% and > 100,000 organisms/ml. in 16% of the patients. "Sterile-voided" urines in the intermediate range (10% of patients) contained < 650 organisms/ml. in subsequent catheterized urines. Bacteruria showed no positive correlation with diagnoses, history, symptoms, laboratory findings or routine cultures. Only increasing age and diabetes mellitus were associated with an increased incidence of bacteruria.

It is concluded that quantitative urine cultures are the only satisfactory means for detecting urinary tract infections, and that "sterile-voided" urine cultures in females are practical for this purpose.

The Value and Interpretation of Diagnostic Renal Biopsy; an Analysis of 150 Consecutive Cases

By *George E. Schreiner and Leonard B. Berman.* Department of Medicine and the Renal Laboratory, Georgetown University Hospital, Washington, D. C.

One hundred and fifty consecutive diagnostic renal biopsies have been analysed. The major indications for renal biopsy were found to be the nephrotic syndrome, multiple renal diagnoses, acute renal insufficiency, chronic pyelonephritis, glomerulonephritis, toxemia of pregnancy, collagen disease and miscellaneous renal disorders. Contraindications were bleeding abnormality, fulminating uremia, unilateral kidney, anuria, renal abscess, perinephritis, malignant hypertension and imminent post mortem. Renal tissue was obtained in 96% of this series and was satisfactory for diagnosis in 91%. The median section contained 13 glomeruli; the mean number was 16 per section. Severe bleeding occurred in 8 patients, urologic consultation was necessary in 4 and surgical intervention in 2. The clinical diagnosis, renal laboratory diagnosis, hospital pathologist's diagnosis, Armed Forces Institute of Pathology

diagnosis and postmortem diagnosis where available, were independently recorded in this series. Renal biopsy, when expertly employed and carefully interpreted, is a valuable diagnostic procedure and a major advance in the management of organic renal disease.

MUSCLE

Excretion of Intravenously Administered Creatine by Healthy and Myopathic Humans

By *Nikos G. Bourdakos.* University of Oklahoma School of Medicine, Oklahoma City.

Although the oral creatine tolerance test has long been in use for the evaluation of myopathies, there is no systematic published information on the occurrence of creatinuria following an intravenously administered creatine load in normal and myopathic subjects. Nor are the renal mechanisms for excretion of creatine or the relation of creatinuria to blood levels clearly understood. Pitts has suggested that there is a renal Tm for creatine while Lillienthal and co-workers, using orally administered creatine, found evidence of variability of renal tubular absorption.

The present study was undertaken to determine the effects of a large intravenous load of creatine in normal and myopathic subjects. The findings led to the reevaluation of the renal mechanism involved.

In the first portion of the study, 0.03 Gm./Kg. creatine hydrate was administered intravenously to 15 patients with various forms of muscular dystrophy with and without spontaneous creatinuria, and compared with a control group of healthy subjects. In both groups serum creatine fell exponentially when plotted on semi-log paper against time. The time required to reach one-half initial concentration was 35–45 minutes. Red cell creatine remained unchanged throughout the test as did serum and urinary creatinine concentrations. The per minute excretion of creatine fell in a stepwise pattern and in both groups creatinuria reflected the concentration of creatine in the serum. Applying the standard procedure the Tm for creatine was determined to lie between 1.1–1.5 mg./min. The creatine load did not enhance urinary excretion of creatinine in either normal or dystrophic humans.

It was evident that spontaneous creatinuria was not a uniform feature of muscular dystrophy. No difference was observed between normal and dystrophic subjects in the way in which creatine was excreted.

In acute experiments the tissue uptake of creatine did not appear to contribute differently to the slope of the excretory curve in either group.

NEOPLASTIC DISEASE

Isolation of Tumor Antiphospholipids from Animal Sera

By *Anwar A. Hakim.* Department of Medical Research, National Children's Cardiac Hospital and Department of Microbiology, University of Miami, Miami.

Phospholipids were isolated and crystallized from DBA-induced transplantable sarcoma (Rd/$_3$), Walker carcinoma, and Sarcoma 180 in rats, and from spontaneous mammary carcinoma in mice.

Rabbits were injected with isolated tumor phospholipids. Sera from these rabbits and from tumor-bearing rats showed strong combining power, with the tumor phospholipids by chromatography, electrophoresis, and Kahn quantitative flocculation tests. These findings demonstrated the relationship of the tumor phospholipid to antigen-antibody development.

Unidimensional ascending chromatography, with barbiturate buffer pH 8.6 and ionic strength 0.05 was employed. Paper ionophoresis was accomplished in an apparatus where the filter paper was sandwiched between two glass plates as described by Tiselius, using the same buffer as for chromatography, at a potential of 200 volts across the filter paper for 48 hours. Chromatography and ionophoresis were carried out in the cold room at 0°C. Quantitative Kahn flocculation tests were conducted according to standard technics.

The antiphospholipids were found and identified in the gamma globulin fractions of the serum of rats bearing the tumor and, in the same fashion, of the sera of the immunized rabbits.

The tumor phospholipid-antiphospholipid com-

plex was separated by ionophoresis on paper. The complex was eluted and split into phospholipid and antiphospholipid (protein in nature) by weak alkali, or by alkaline potassium chloride, and the two components were isolated separately by ionophoresis, according to the same technic as above.

These investigations render support to the hypothesis that tumors contain specific antigenic material, capable of evoking antibody formation, manifested by the presence of such specific antibodies in the gamma globulin fraction of the sera of both tumor-bearing and "immunized" animals.

The specific antigenic materials were acetal phospholipids.

RESPIRATORY SYSTEM

An Evaluation of The Mechanical Characteristics and the Physiologic Effects of Intermittent Pressure Breathing Devices

By *Nancy Wu, William F. Miller and Ivan E. Cushing.* Cardiopulmonary Laboratory, Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

Increasing clinical application of mechanical breathing devices necessitates understanding their function. Alterations in mechanical properties of the respiratory apparatus in patients with pulmonary disease would be expected to alter the performance of respiratory assistors.

Simultaneous recordings of air flow, volume, esophageal and airway pressures were made. FRC was determined for each set of measurements.

The pressure-flow characteristics of breathing devices are of two types. Type I: flow decreases essentially linearly as resistive pressure approaches limiting values. Type II: flow is maintained high until resistive pressure approaches the present level; then flow ceases abruptly, effecting a smaller tidal flow for any given airway pressure.

In relaxed normal subjects, increasing airway pressure increases tidal volume, limited by increasing chest wall resistance with no change in lung compliance.

In patients with emphysema having markedly increased viscous resistance, tidal volume is limited by decreasing lung compliance owing to air trapping with elevation of the respiratory midposition near the elastic limit of lung expansion. Thoracic cage compliance remains unchanged since the volume at which the chest wall resists further expansion was not reached.

Poliomyelitis patients respond in two ways to increasing airway pressure: 1. Measured compliance increases since larger portions of lung become ventilated. Thoracic cage compliance decrease depends on the duration of the disease. 2. Lung compliance decreases until critical opening pressure of resistive portions of lung is exceeded; thus, only small increases in tidal volume occurrs until airway pressure becomes very high. In some chronic cases, the response is that of emphysema patients.

The extent to which intrathoracic pressure reflects positive airway pressure depends on these same relationships between mechanical properties of the apparatus and the patient's respiratory system.

Again, knowledge of the nature of altered function responses provides the only logical basis for application of a therapeutic procedure.

The Influence of Pulmonary Capillary Blood Flow upon Alveolo-Capillary Gas Exchange

By *Peter C. Luchsinger, Georges F. McCormick and Kenneth M. Moser.* Cardiopulmonary Function Laboratory, D. C. General Hospital, and the Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

The low pressure, low resistance characteristics of the pulmonary circuit are dependent upon its marked expansibility of "reserve capacity." Such distensibility allows vascular resistance to fall as pulmonary blood flow rises, thus preserving normal pulmonary artery pressure.

However, if this "reserve capacity" be markedly diminished, e.g., by extensive pulmonary resection or fibrosis, the remaining vessels reach their maximum expansible capacity at relatively low levels of pulmonary blood flow, with resultant fixation of pulmonary vascular resistance. If pulmonary flow is then further increased, pulmonary artery pressure and right ventricular work rise sharply, and a diffusion insufficiency for oxygen develops. This latter effect reflects the fact that, in the face of a fixed resistance, the lungs can accommodate further blood only by accelerating flow. Consequently, "contact time" between capillary blood and alveolar air is diminished, and alveolo-capillary oxygen tensions do not equilibrate. Thus, despite

normal alveolar oxygen tensions, arterial hypoxemia develops, i.e., an abnormal A-a gradient appears.

In the patients presented here, prior study (1) ruled out the presence of alveolar hypoventilation, shunt or distribution defects; (2) demonstrated a diffusion insufficiency for oxygen at rest, exaggerated by exercise. Simultaneous cardiac catheterization and pulmonary function testing was then done. At rest, each patient demonstrated a high pulmonary vascular resistance, pulmonary hypertension, an abnormal A-a gradient and arterial hypoxemia. These findings indicated that the pathologic reduction of vascular bed had been so extensive that even resting pulmonary blood flow exceeded vascular 'capacity.'

When cardiac output was lowered by hexamethonium infusion, pulmonary artery pressure and right ventricular work decreased, despite a fixed resistance. Simultaneously, the A-a gradient narrowed and arterial hypoxemia lessened, reflecting the longer alveolo-capillary contact time permitted by decreased pulmonary blood flow.

Demonstration of these relationships at various levels of cardiac output re-emphasizes that the physiologic block offered by a reduced alveolo-capillary contact time is the central feature in the genesis of diffusion insufficiency for oxygen (Rossier et al.).

Venous Pressure Studies in Pulmonary Emphysema

By *J. K. Alexander, J. Mise and E. W. Dennis.* Department of Medicine, Baylor University College of Medicine, and the Cardiac Clinic Laboratory, Jefferson Davis Hospital, Houston.

Simultaneous measurements of pressure in the brachial artery, right atrium and both vena cavae have been made in 5 normal subjects and in 6 patients with pulmonary emphysema during spontaneous respiration, cough, and performance of the Valsalva maneuver. Mean pressure gradient between superior cava and atrium was comparable in the normal and emphysematous groups, but the mean pressure gradient between inferior cava and atrium was many times the normal in emphysematous subjects. The elevated pressure gradient between inferior cava and atrium in emphysema was wholly due to changes occurring during the inspiratory phase of respiration, when the gradient averaged more than 12 mm. Hg, as compared to the normal of less than 1 mm. Hg. In the emphysematous group the observed changes in venous pressure were well correlated with changes in intrathoracic (intraesophageal) pressure, the pattern of respiratory action and, to a lesser extent, with the severity of the disease process as indicated by standard pulmonary function tests. Implications of these data in terms of venous return from upper and lower portions of the body in emphysematous subjects were studied. Representative records indicating the responses in arterial and venous pressure in normal and emphysematous subjects relative to the pattern of respiratory action were also taken.

Ventilatory Function in Pulmonary Sarcoidosis

By *C. Morrison, J. Fulton and J. B. Hickam.* Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

Pulmonary sarcoidosis often produces severe, permanent impairment of lung function. Divergent observations have been reported on the extent to which sarcoidosis and subsequent scarring may bring about diffuse bronchiolar narrowing and a functional picture resembling obstructive pulmonary emphysema. It is the purpose of this report to summarize our experience with ventilatory function in 29 patients with pulmonary sarcoidosis, with particular reference to the nature of the functional impairment.

All cases had a lymph node or skin biopsy compatible with sarcoidosis, and all had roentgenologic evidence of pulmonary involvement. In 6 cases the only definite roentgenologic change was bilateral parenchymatous infiltration. Measurements of pulmonary function included maximum breathing capacity, the conventional lung volume determinations, and measurements of intrapulmonary gas mixing by an open-circuit helium method. Results were compared with normal values and with values obtained on patients with pulmonary tuberculosis and with obstructive pulmonary emphysema.

The group with sarcoidosis showed a reduction of total lung volume to about two-thirds of normal (4.01 L., S.D. $\pm$ 1.04). The ratio of residual to total lung volume was slightly increased (38% $\pm$ 12), as in emphysema, but the absolute values of residual capacity (1.49 $\pm$ .65 L.) and functional residual capacity (2.43 $\pm$ .84 L.) was normal to low. Maximum breathing capacity (75 $\pm$ 34 L./min.) and vital capacity (2.62 $\pm$ .88 L.) were both approximately 70% of predicted normal, yielding a normal ratio of the two, or "air velocity index." Intrapulmonary gas mixing was not abnormally delayed as in emphysema. The rate at which a test gas was cleared from the lungs was significantly faster than in the group with pulmonary tuberculosis. It is concluded that the present group showed considerable reduction in lung volume but no significant evidence of diffuse bronchial obstruction and little or no impairment of intrapulmonary gas mixing. The func-

tional picture did not resemble that of obstructive pulmonary emphysema.

Anesthesia Research. I. Comparison of "Usual" vs. Mechanical Inflation

By *John O. Dampeer, Jr., Don M. Turner, James D. Hardy and Glace E. Bittenbender.* Department of Surgery, University of Mississippi, Jackson.

Cardiac arrest constitutes a major cause of mortality during the operation itself, and most instances of this complication result from inadequate pulmonary ventilation. The objective of the present study was to compare arterial pH, pCO_2, and oxygen saturation values obtained in patients on the automatic Jefferson ventilator with those ventilated by spontaneous respiration, supplemented by manual compression of the gas bag at the discretion of the anesthetist. During the early part of the study the purpose of the arterial blood sampling was unknown to the nurse anesthetists, though later in the series this secrecy could no longer be maintained.

The blood gas analyses were performed largely with the Van Slyke manometric apparatus, and the pH measurements with a Beckman instrument. Thus far, 30 patients have been studied before, during, and following operation. Fewer instances of inadequate ventilation were encountered in the "ventilator" group than in the "usual" group: in three of the latter, serious acidosis and diminished arterial oxygen saturation occurred. Yet, these important individual variations were not especially apparent when the curves derived from averaged values in the two groups were compared. It is concluded that while a competent anesthetist can achieve adequate anesthesia and ventilation without an automatic respirator, the proper use of such an instrument adds to the safety factor.

Studies Relative to the Function of the Human Sympathetic Nervous System in Relation to the Lung

By *Osler A. Abbott and Tet H. Pang.* Department of Surgery, Emory University School of Medicine, Atlanta.

This study consists of an attempt to define the action of the human sympathetic nervous system on the lung. The studies have been carried out at the time of open thoracotomy in patients with different types of intrathoracic disease. Particular interest has been paid to those patients in whom thoracic surgery has been performed for nonpulmonary lesions. Studies concerned with direct instillation of novacaine subpleurally about the sympathetic nerves and subsequent studies with the nerve trunks being exposed have been carried out. In addition, methods for blocking the sympathetic nervous system as it is carried into the lung by the adventitia of the primary pulmonary artery have been developed. The initial studies were related to the effect of sympathetic nervous system block upon pulmonary artery pressure; more recent studies have been related to the effect upon the oxygen and carbon dioxide content of the pulmonary artery and vein as well as to the pH of blood in these two areas. Initial studies would suggest that blocking the sympathetic nervous system to the lung, even in its normal state, tends to increase pulmonary artery pressure. This presents a serious question of the advisability of sympathectomy as it is now being suggested for either primary pulmonary arteriosclerosis or for reverse flow patent ductus.

Injuries to Main Stem Bronchi

By *James D. Hardy.* Department of Surgery, University of Mississippi, Jackson.

Bronchial injuries are increasingly encountered. This study presents two spectacular illustrative cases. The first patient was an 18-year-old girl who sustained head injury and fractures of long bones and of both first ribs. Left pneumothorax was treated and the lung was re-expanded briefly but then collapsed again. Bronchoscopy and bronchogram revealed occlusion of left main stem bronchus 5 cm. from carina. At thoracotomy, 3 months after injury, the ends of the bronchus, separated by 3 cm., were debrided and successfully anastomosed.

The second case was a 34-year-old male who also sustained bilateral first rib fractures in a car wreck. Here, however, the right main bronchus was kinked due to loss of rigidity from multiple fractures of adjacent cartilages. Stability and patency of the bronchus was achieved at operation, and thereafter the lung remained expanded. To our knowledge, this is the first description of this phenomenon of kinked bronchus.

THERAPEUTICS

Pharmacodynamics of Two Trifluoro Derivatives of Phenothiazine; A Study of Antiemetic Activity and Renal Responses

By *Paul K. Conner, Wilson Fraser, Sam I. Kinard, Hugh Bennett and John H. Moyer.* Baylor University College of Medicine, Houston.

Chlorpromazine has ushered in a new era for agents that are effective in arresting nausea and vomiting. However, chlorpromazine exhibits many pharmacologic properties, both useful and detrimental, that are unrelated to the antiemetic effects of this agent. Recently, we have studied two new fluoride derivatives of phenothiazine: SKF 4648, the compound that is a trifluoro-methyl derivative of promazine and SKF 5019, also a trifluoro derivative of phenothiazine. The latter in addition has a piperazine ring, which usually increases the potency (as well as toxicity) of these agents. Laboratory observations have been made on the renal response to these agents, including glomerular filtration rate, renal blood flow, and water and electrolyte excretion. In addition, the antiemetic properties and cardiovascular effects of these drugs were observed. Clinical observations on antiemetic properties, cardiovascular effects, evidence of hepatic and renal toxicity were made. The studies indicate that these compounds have no effect on renal hemodynamics or on water and electrolyte excretion. Both compounds were 8 to 10 times as potent as chlorpromazine in protecting animals against apomorphine emesis. The drugs produced less adrenergic blockade than chlorpromazine and, consequently, less hypotension. Clinically, the drugs were 8 to 10 times as potent as chlorpromazine in protection against nausea and vomiting due to numerous causes. There has been no evidence of renal, hepatic, or cardiovascular toxicity to date. SKF 4648 is superior to SKF 5019 as an antiemetic because of psychiatric effects associated with the latter agent. These include insomnia, depression, withdrawal phenomena, and inability to think clearly.

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